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(For references see pages 460–461)

Age at which symptoms appear	Genetics	Reference
From birth Childhood Up to 30 years or later	(a) Recessive (b) Recessive (c) Uncertain	3
1–2 years Late childhood or adulthood	(a) Recessive (b) Uncertain	4
Usually early childhood	Complex	5
In childhood Cards	Usually dominant	5, 6

et al. (Eds.), *The Metabolic Basis of Inherited* l, New York, 1966, page 539.
ANBURY et al. (Eds.), *The Metabolic Basis of* Graw-Hill, New York, 1966, page 429.
ore), 45, 77 (1966).

is excrete only small amounts of 17- and they show hardly any increase in rticosterone causes sodium retention, se of the arterial hypertension seen in

ysteroid dehydrogenase is rare; this gesterone from pregnenolone. As with on, the result is loss of salt in the urine, differs from inborn adrenal hyperplasia affects differentiation of the external

steroid 17α-hydroxylase has been re- cortex produced excessive amounts of rticosterone, with consequent arterial

orn adrenal hyperplasia are probably utosomal recessive gene.

al Gland, Lea & Febiger, Philadelphia, 1961; ANBURY et al. (Eds.), *The Metabolic Basis of* Graw-Hill, New York, 1966, page 635.
5, 1946 (1966).

Vitamin A[1,2]

Chemistry[3]

Vitamin A and the carotenes are soluble in fats, insoluble in water, and readily oxidized. In the absence of oxygen they are stable to acids, alkalis and heat. On the *cis-trans* isomerism of the carotenes and vitamin A see ZECHMEISTER[4]. For structure and properties of vitamin A and related compounds see the table on pages 458–459.

Assay

Biological[5]. Mainly by the standardized growth test on vitamin A-deficient rats.

Chemical[6]. Spectrophotometrically in pure solution (vitamin A at 328 nm, carotenes at ca. 450 nm) or colorimetrically, for instance with antimony trichloride (CARR-PRICE reaction); in biological material chromatographically after suitable extraction.

Units

Vitamin A. 1 International Unit (IU) = 0.344 μg *all-trans* vitamin A_1 acetate = 0.300 μg *all-trans* vitamin A_1. 1 US Pharmacopeia (USP) Unit = 1 International Unit.

Carotenes. 1 International Unit (IU) = 0.6 μg β-carotene, equivalent in activity to 1 IU vitamin A.

Biogenesis[7,8]

The carotenes are synthesized by the higher plants, algae and photosynthetic bacteria and are found in concentrated form in the chloroplasts. Acetate is converted by condensation and decarboxylation into isopentenyl pyrophosphate, from which a C_{20} terpenol pyrophosphate arises by condensation. This substance gives rise by further condensation to a carotenoid precursor with 40 C-atoms, probably phytoene. The various carotenes arise by dehydrogenation, cyclization, isomerization, hydration and hydroxylation. Carotenes with a β-ionone ring are broken down in the animal organism to vitamin A, more probably by fission in the middle of the chain than by successive β-oxidation from the end of the isoprenoid chain[9]. In the liver oils, vitamin A is present in the esterified form.

Intake and excretion

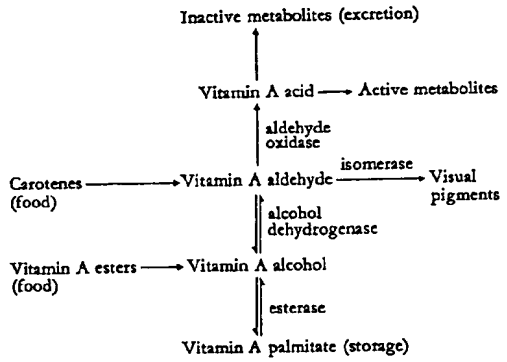
In the USA the daily diet contains ca. 7500–10000 IU (2.3–3 mg) of vitamin A[10,11]. About a half of the apparent vitamin intake is in the form of the provitamin.

The vitamin A esters are hydrolysed in the lumen of the duodenum, probably by esterases of the pancreatic juice. Bile is necessary for absorption of the carotenes but not of vitamin A; the absorption of the carotenes is promoted by fats. In the wall of the duodenum and upper jejunum the carotenes are broken down to vitamin A, with vitamin A aldehyde as an intermediate product[9,12]. In the intestinal wall vitamin A is probably mainly esterified with palmitic acid, the ester being transported by the lymph[13,14]. The carotenes are less easily absorbed than vitamin A and part of those ingested appears in the faeces. Under favourable conditions the biological activity of β-carotene amounts to about half that of vitamin A.

The vitamin A level in blood is at its highest about 4 hours after giving the vitamin[2]. The mean normal levels in serum are 850 μg carotene/l and 324 μg vitamin A/l (see page 609). In the serum, vitamin A is normally present to the extent of about 90% as the alcohol, the remainder as the ester; shortly after intake of vitamin A, however, the proportion of ester is increased[15]. Freshly absorbed vitamin A esters are transported by the lipoproteins S_f 10–400. Vitamin A alcohol is probably bound to albumin in the serum[14]. In breast milk vitamin A is about 90% esterified.

Vitamin A is stored in the liver, the carotenes mainly in the fatty tissues. About 90% of the whole vitamin A of the body is stored in the liver; the liver reserves (up to 300 μg vitamin A per gramme liver or more[16]) are sufficient to meet the body's requirements of the vitamin for one year or more[2]. These reserves, however, are rapidly used up in infections, hyperthermia and poisoning[2]. 90–95% of the vitamin A in the liver is present as palmitate, the remainder as aldehyde and alcohol[14,15]. When any organ requires vitamin A, the esters in the liver are hydrolysed and the free alcohol transported by the blood to where it is required. In the tissues, particularly in the liver, vitamin A alcohol and aldehyde are rapidly oxidized to vitamin A acid; this substance is not stored, however, but rapidly broken down[8,17]. An active metabolite of vitamin A acid has recently been identified but its composition is unknown[16]. After administration of tagged vitamin A to rats, radioactivity can be detected in the bile, urine and faeces[8]; the presence of vitamin A and/or its metabolites in bile points to enterohepatic circulation of the vitamin[18].

Metabolism of vitamin A



Function

Vitamin A is of great importance for maintenance of health and life, normal growth, the visual process and reproductivity. It appears to be necessary for the stability of the lipoprotein membrane of the cell and of the subcellular particles[19].

Vitamin A alcohol can be converted in the body to the aldehyde and acid and has a specific effect on the ability to reproduce. Vitamin A aldehyde is the prosthetic group of the visual pigments. Vitamin A acid, while very active in maintaining growth, is not capable of maintaining reproductivity.

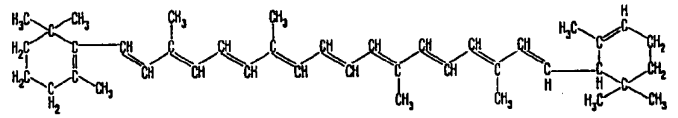
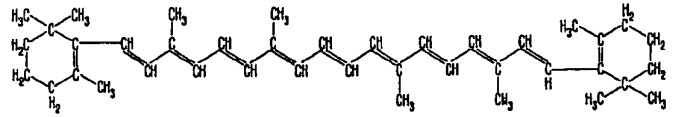
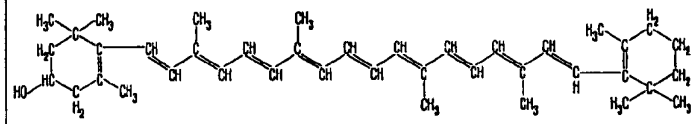
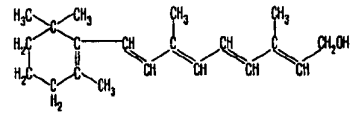
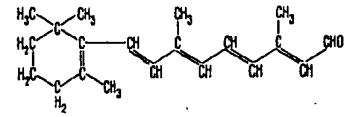
Metabolic functions of vitamin A[20]

Active form	Biochemical reaction	Clinical effect
Vitamin A alcohol or other active form	Unknown	Reproduction in both sexes
Vitamin A aldehyde	Reaction with opsin	Visual process
Vitamin A acid or other active form	Liberation of proteolytic enzymes	Breakdown of cartilage
	Synthesis of mucopolysaccharides	Stimulation of mucous secretion in the epithelium
	Synthesis of corticosterone	Lesions in the adrenal cortex, interference with gluconeogenesis in deficiency states

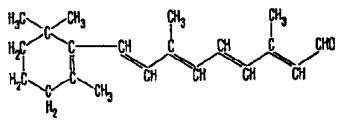
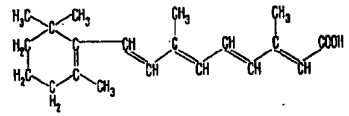
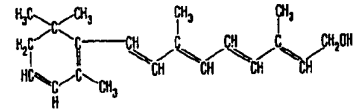
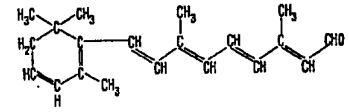
The vitamin A aldehydes retinal and dehydroretinal form together with the protein opsin the light-sensitive pigments in the rods (night vision) and cones (day and colour vision) of the retina. The four main types of pigment[21,22] are rhodopsin (retinal + rod opsin) in terrestrial and aquatic animals, iodopsin (retinal + cone opsin) in terrestrial animals, porphyropsin (dehydroretinal + rod opsin) in fresh-water animals, and cyanopsin (dehydroretinal + cone opsin). Absorption of light by the visual pigments causes transformation of the 11-cis isomers of retinal or dehydroretinal into the *all-trans* form. The potential difference induced by this isomerization is responsible for the registration in the brain of the visual impressions from the rods and cones. 11-*cis*-Retinal is not only the prosthetic group of the visual pigment in the rods but also of the red- and green-sensitive pigments in the cones of the human eye[23]. The *cis-trans* isomerization of retinal proceeds as follows[21]:

(continued on page 460)

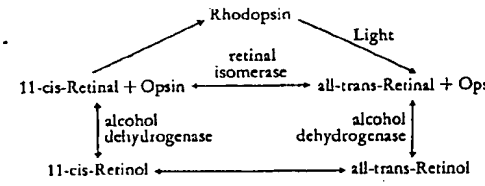
Structure and properties of vitamin A and related compounds

Names*	Formula and mol. wt.	Structure	Physical properties	Occurrence	Relative activity
α-Carotene	$C_{40}H_{56}$ 536.89		Violet to red crystals M.p. 187 °C (benzene/methanol)	Palm oil, mountain ash berries	50
β-Carotene	$C_{40}H_{56}$ 536.89		Violet to red crystals M.p. 180 °C	Plants, fruits	100
Cryptoxanthene (3-hydroxy-β-carotene)	$C_{40}H_{56}O$ 552.89		Red platelets M.p. 158 °C	Maize	50
Vitamin A_1 (*all-trans*) (retinol*, axerophthol)	$C_{20}H_{30}O$ 286.46		Yellow prisms M.p. 62–64 °C	Liver of marine fish	100
9-*cis*-Vitamin A_1 (*iso-a*)	$C_{20}H_{30}O$ 286.46	As *all-trans* vitamin A_1 but with double bond at C-9 in *cis* configuration	Yellow prisms M.p. 82 °C	–	21
11-*cis*-Vitamin A_1 (*neo-b*) (11-*cis*-retinol)	$C_{20}H_{30}O$ 286.46	As *all-trans* vitamin A_1 but with double bond at C-11 in *cis* configuration	Orange-yellow oil	Retina	23
13-*cis*-Vitamin A_1 (*neo-a*)	$C_{20}H_{30}O$ 286.46	As *all-trans* vitamin A_1 but with double bond at C-13 in *cis* configuration	Yellow prisms M.p. 58 °C	Fish liver	75
Vitamin-A_1 aldehyde (*all-trans*) (retinal*, retin-aldehyde*, β-retinene, retinene$_1$)	$C_{20}H_{28}O$ 284.45		Orange prisms M.p. 58 °C	Citrus fruits, green vegetables, liver	91

(*iso-a*)	286.46		Yellow prisms M.p. 82 °C	–	21
11-*cis*-Vitamin A_1 (*neo-b*) (11-*cis*-retinol)	$C_{20}H_{30}O$ 286.46	As *all-trans* vitamin A_1 but with double bond at C-11 in *cis* configuration	Orange-yellow oil	Retina	23

13-*cis*-Vitamin A_1 (*neo-a*)	$C_{20}H_{30}O$ 286.46	As *all-trans* vitamin A_1 but with double bond at C-13 in *cis* configuration	Yellow prisms M.p. 58 °C	Fish liver	75
Vitamin-A_1 aldehyde (*all-trans*) (retinal*, retinaldehyde*, β-retinene, retinene$_1$)	$C_{20}H_{28}O$ 284.45		Orange prisms M.p. 58 °C	Citrus fruits, green vegetables, liver	91
11-*cis*-Vitamin-A_1 aldehyde (*neo-b*) (11-*cis*-retinal)	$C_{20}H_{28}O$ 284.45	As *all-trans* vitamin-A_1 aldehyde but with double bond at C-11 in *cis* configuration	Orange prisms M.p. 64 °C	Eyes of crustacea	48
Vitamin-A_1 carboxylic acid (*all-trans*) (retinic acid*)	$C_{20}H_{28}O_2$ 300.44		Yellow needles M.p. 179 °C	Tissues?	~65
Vitamin A_2 (3-dehydroretinol*)	$C_{20}H_{28}O$ 284.45		Yellow needles M.p. 63–65 °C	Liver of freshwater fish	40
Vitamin A_2 aldehyde (3-dehydroretinal*, 3-dehydroretinaldehyde*, α-retinene, retinene$_2$)	$C_{20}H_{26}O$ 282.43		Orange-red prisms M.p. 78 °C	Retina in fish	

* Trivial names recommended by the Commission on Biochemical Nomenclature of the International Union of Pure and Applied Chemistry and the International Union of Biochemistry [*Biochim. biophys. Acta (Amst.)*, 107, 1 (1965)].



Vitamin A and carotene in their protein-bound form are thought to participate in an analogous manner in the sense of smell[24].

Requirements and deficiency symptoms

The requirement of vitamin A is proportional to the body weight. Daily requirements in health allowing for some reserve are 2500 IU vitamin A, 4000 IU carotene in fats, 7500 IU in green vegetables or 12000 IU in boiled carrots[26]. In 10- to 15-year-old boys 1700 IU vitamin A are sufficient to maintain a plasma level of 300 µg/l[27]. For infants from birth to 5 months it is assumed that exclusive breast-feeding can provide sufficient vitamin A[25]. For recommendations of official bodies see the tables on pages 493–494.

Good sources of vitamin A are the fish oils (cod 1000, herring 5000, halibut and tunny 50000–100000 IU/g), liver, milk fat and egg yolk; green vegetables and carrots are rich in carotenes. See also pages 499–515.

Causes of vitamin A deficiency[2] are inadequate dietary intake, impaired absorption (fat deficiency) or storage, disturbances in the conversion of carotene into vitamin A, or rapid depletion of the body's reserves. Impairment of absorption or storage is seen in coeliac disease, cystic fibrosis of the pancreas, ulcerative colitis, pancreatectomy, obstruction of the biliary ducts and cirrhosis of the liver. Conversion of carotenes may also be impaired in diabetes and hyperthyroidism. Some infections result in disappearance of vitamin A from the blood. The typical lesions of vitamin A deficiency[28] are night blindness, xerosis or keratinization of various membranes (particularly xerophthalmia) and the formation of defective bony tissue and dentine during growth. The most sensitive test for vitamin A deficiency is measurement of dark adaptation of the eye[26]; determination of vitamin A concentration in the serum is less reliable since the serum level does not fall until the body's reserves are fully depleted[2]. In many countries of South America, Asia and Africa xerophthalmia is still one of the commonest causes of blindness in children[29,30]. Other manifestations of vitamin A deficiency are Bitôt's spots on the conjunctiva and roughness of the skin due to hyperkeratosis of the hair follicles. In animals vitamin A deficiency has serious effects in pregnancy and is a cause of infertility and congenital deformities[31].

Vitamin A deficiency symptoms in pregnancy and at various ages (modified from McLaren and Halasa[30])

	Cause of deficiency	Symptoms
Pregnancy	Dietary carotene deficiency, increased requirement, depletion following repeated pregnancies	Low plasma level of vitamin A, low liver reserves, xerophthalmia (rare), Bitôt's spots (occasionally)
Foetus		Low liver reserves, xerophthalmia (rare), abortion (?), congenital deformities (?)
Up to 12 months	Inadequacy of breast milk, low vitamin A content of breast milk, bottle feeding, infections	Low plasma level of vitamin A, depletion of liver reserves, xerophthalmia (fairly common), Bitôt's spots (rare)
Up to 5 years	Breast feeding continued too long, dietary deficiency, infections	Commonest cause of conjunctival xerosis, xerophthalmia, Bitôt's spots (occasionally)
School age	Dietary deficiency of carotene, vitamin A, fats and proteins	Conjunctival xerosis and Bitôt's spots (main symptoms), night blindness, follicular hyperkeratosis (occasionally)
Adults	Dietary deficiency, infections, cirrhosis of the liver, pancreatic disease	Night blindness (main symptom), Bitôt's spots (occasionally), xerophthalmia (rare), follicular hyperkeratosis (occasionally)

Treatment and toxicity

Deficiency symptoms should be treated by giving vitamin A in doses of up to 25000 IU (corresponding to ca. 30 ml liver oil). Xerophthalmia calls for initially higher doses (5000 IU/kg body weight daily for 5 days)[29,30]. When liver oils are given in large doses, vitamin D may be ingested in toxic amounts, even though it has been shown that vitamin A in large doses diminishes the toxic effect of vitamin D[32].

Protracted treatment with high doses of vitamin A (for instance 100000 IU or more per day in children) may result in toxic symptoms such as anorexia, alopecia, affections of the skin and mucosa, swelling of the bones and diaphyses of the limbs, anaemia, enlargement of the liver and spleen and headache. All these symptoms are reversible and disappear rapidly on cessation of the treatment[2]. In children overdosage of vitamin A may interfere with bone development and lead to premature fusion of the epiphyses[33].

In some countries vitamin A is given prophylactically to newborn children and infants in daily doses of 7500–10000 IU. Because of the danger of possible intoxication, however, it is better to restrict prophylactic doses to 2500 IU per day[34].

Acute vitamin A intoxication has been observed following the consumption of polar bear liver, which contains 20000 IU per gramme[35,36]; whale liver contains 4400 IU vitamin A per gramme, swine's liver only 100–150 IU per gramme[36].

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35 Rodahl and Moore, *Biochem. J.*, 37, 1
36 Notes, *Nutr. Rev.*, 19, 318 (1961).

Vitamin D[1,2]

Chemistry[1,3]

The D vitamins are odourless c
solvents, insoluble in water, and o
show a wide absorption band at 2
properties of vitamin D and relate
pages 462–463.

Assay

Biological[1]. By its preventive or cu
experimental rickets.

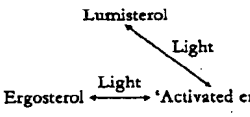
Chemical[4]. Spectrophotometrical
the individual D vitamins cannot be
with antimony trichloride, in the pr
matographic separation. The individ
by column, paper or thin-layer chro

Units

1 International Unit (IU) = 0.025
Pharmacopeia Unit = 1 Internatio
Standard for Vitamin D_3, page 763
equivalent to 1 mg of standardized
in a vegetable oil is now obsolete.

Biogenesis

Vitamin D_3 occurs solely in the
photochemically from 7-dehydroch
mal provitamin). 7-Dehydrocholest
by a reaction that also takes place in
vitamins of known constitution and f
at wave lengths of 275–300 nm are
terol, D_4 from 22-dihydroergosterol
D_6 from 2-dehydrositgmasterol and
The conversion of provitamin D
occurs as follows[5]:



Products of over-irradiation ←

In fish liver oils vitamin D is partl
min D in fish is not known. Plants c
antirachitic effect (ketone 250).

Intake and excretion

In man, vitamin D is ingested in fo
from 7-dehydrocholesterol in the sk
The vitamin formed in the skin is ve
ported almost quantitatively to the
appears to be necessary for the abso
foods. In rats, the vitamin is partly e
is transported by the lymph[9]. A sin
served in man[10]. In children the blo
adults 700–3100 IU/l[36]. With a dail
IU, the blood level in adults rises t
blood, the vitamin is transported bo
bumin[13]. It is taken up by and meta
present in other tissues such as the

(For references see page 464)

[28] Moore, T., *Vitam. and Horm.*, 18, 499 (1960).
[29] McLaren, D. S., *Nutr. Rev.*, 22, 289 (1964).
[30] McLaren and Halasa, *Postgrad. med. J.*, 40, 711 (1964).
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[35] Rodahl and Moore, *Biochem. J.*, 37, 166 (1943).
[36] Notes, *Nutr. Rev.*, 19, 318 (1961).

Vitamin D[1,2]

Chemistry[1,3]

The D vitamins are odourless crystals, soluble in fats and fat solvents, insoluble in water, and only slightly photosensitive. All show a wide absorption band at 260–290 nm. For structure and properties of vitamin D and related compounds see the table on pages 462–463.

Assay

Biological[1]. By its preventive or curative effect on young rats with experimental rickets.

Chemical[4]. Spectrophotometrically in pure solution at 265 nm; the individual D vitamins cannot be distinguished. Colorimetrically with antimony trichloride, in the presence of vitamin A after chromatographic separation. The individual D vitamins can be separated by column, paper or thin-layer chromatography.

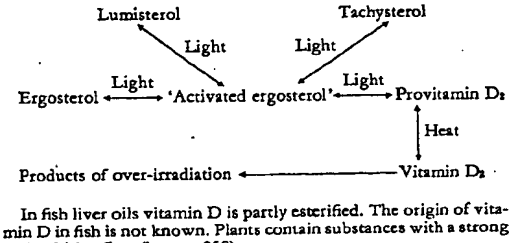
Units

1 International Unit (IU) = 0.025 μg crystalline vitamin D_3. 1 US Pharmacopeia Unit = 1 International Unit (see also International Standard for Vitamin D_3, page 763). The earlier international unit equivalent to 1 mg of standardized irradiated ergosterol dissolved in a vegetable oil is now obsolete.

Biogenesis

Vitamin D_3 occurs solely in the higher animals and is formed photochemically from 7-dehydrocholesterol (provitamin D_3, animal provitamin). 7-Dehydrocholesterol is formed from cholesterol by a reaction that also takes place in the intestinal tissues[5]. Other D vitamins of known constitution and formed by ultraviolet irradiation at wave lengths of 275–300 nm are the following: D_3 from ergosterol, D_4 from 22-dihydroergosterol, D_5 from 7-dehydrositosterol, D_6 from 2-dehydrostigmasterol and D_7 from 7-dehydrocampesterol.

The conversion of provitamin D into vitamin D by irradiation occurs as follows[6]:



In fish liver oils vitamin D is partly esterified. The origin of vitamin D in fish is not known. Plants contain substances with a strong antirachitic effect (ketone 250).

Intake and excretion

In man, vitamin D is ingested in foods in addition to being formed from 7-dehydrocholesterol in the skin under the effect of sunlight. The vitamin formed in the skin is very rapidly absorbed[7] and transported almost quantitatively to the sites where it is required[8]. Bile appears to be necessary for the absorption of vitamin D ingested in foods. In rats, the vitamin is partly esterified during absorption and is transported by the lymph[9]. A similar process has also been observed in man[10]. In children the blood level is 860–2100 IU/l[11], in adults 700–3100 IU/l[36]. With a daily oral intake of 50000–500000 IU, the blood level in adults rises to 90000–130000 IU/l[12]. In the blood, the vitamin is transported bound to the α_3-globulins and albumin[13]. It is taken up by and metabolized in the liver, and is also present in other tissues such as the kidneys, intestine, adrenals and bones[13,14]. 24 hours after giving ^{14}C-tagged vitamin D, 10% of the activity was found in the tissues and 20% in the faeces, the remaining 70% being present as breakdown products[13]. In rats, some vitamin D is excreted in the bile bound to taurine and glycine and then partly reabsorbed. In man, the urine contains no vitamin D as such but up to 2–4% of the radioactivity of ingested tagged vitamin D has been found there[31].

The vitamin D content of the skin exposed to sunlight amounts to ca. 1 IU/cm². In the skin of the human back activation may reach the level of 15 IU/cm². 50–75% of the activity is found in the deeper layers of the epidermis and 25–50% in the parts of the corium adjacent to the epidermis[6].

It has been suggested that the metabolic effect of vitamin D is due not to the vitamin itself but to a metabolite[14]. Such an active metabolite has been found in the nuclear fraction of the intestinal mucosa[32]. Recently a biologically active metabolite of vitamin D_3 has been identified as 25-hydroxycholecalciferol[33].

Function

The activity of vitamin D is closely related to that of parathyroid hormone and of calcitonin, all three factors being necessary for maintenance of calcium balance and a normal serum calcium level[14,16]. Vitamin D may be responsible for formation of the calcium transport system in bone cells; the control of serum calcium concentration by parathyroid hormone depends on the presence of vitamin D, whereas that by calcitonin does not[14]. It has been shown that the physiological expression of the action of vitamin D on intestinal calcium transport and bone mineral mobilization probably involves DNA transcription into messenger RNA and protein synthesis[14]. At the subcellular level, vitamin D causes release of the bound calcium in the mitochondria, probably as a result of its action on oxidative phosphorylation[15].

In rats, vitamin D has been shown to increase calcium absorption mainly in the intestine[17]. A vitamin D-dependent calcium-binding protein has been isolated from the intestinal mucosa of chicks[34]. There is evidence that vitamin D also favours the intestinal absorption of magnesium[18].

Vitamin D is also necessary for the development of normal bone and for the calcification of rachitic bone. The manner in which it acts is obscure but probably the defective calcification of newly formed bone in rickets and osteomalacia is due to a reduction of the calcium phosphate product in the tissue fluid surrounding the osteoid[19]. The increase in the serum citrate level caused by vitamin D is probably due to its effect on calcium metabolism[16].

It is not certain whether vitamin D has any effect on the renal transport of calcium; in any case this effect is small in comparison with that of parathyroid hormone[16]. Vitamin D is thought to favour the tubular reabsorption of phosphate[20].

Requirements and deficiency symptoms

In adults the body's needs of vitamin D are usually met by its own synthesis, provided this is not impaired by lack of daylight, as in night workers, miners, and people living in subpolar regions. During the period of active skeletal growth, in pregnancy and in lactation, there is an increase in both the calcium and vitamin D requirements, so that intake of exogenous vitamin D is advisable. In such cases, oral administration of 400 IU vitamin D per day is adequate[21] (see also pages 493–494).

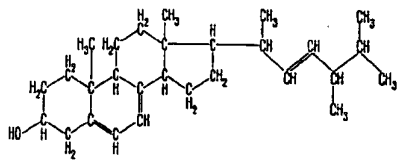
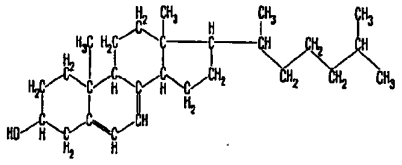
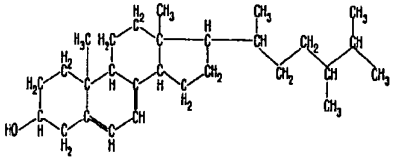
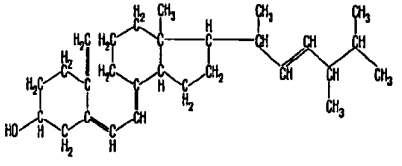
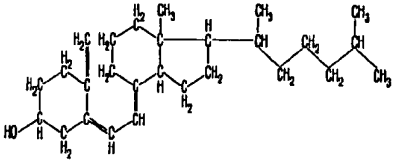
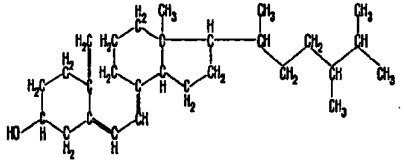
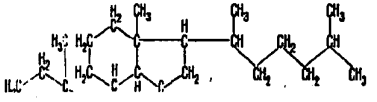
The liver oils are rich in vitamin D: tunny fish 7000–50000 IU per gramme, cod 60–300 IU per gramme; the mammalian liver contains only small quantities of vitamin D, egg yolk 1.5–5 IU per gramme (values from Dam and Søndergaard[1]).

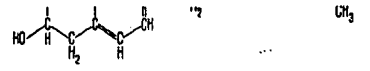
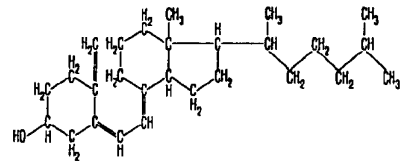
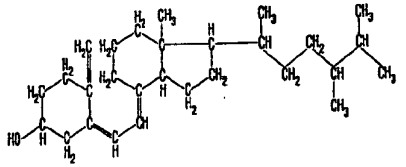
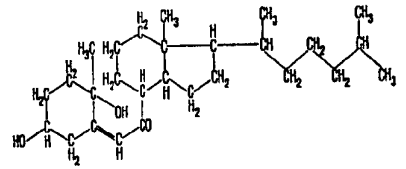
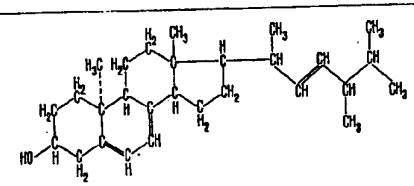
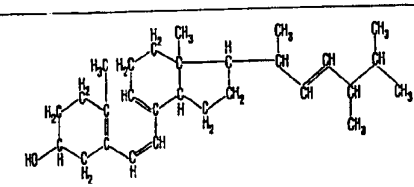
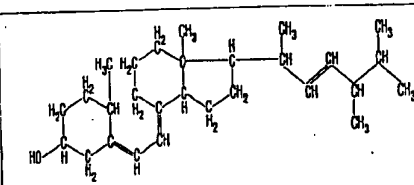
Deficiency of vitamin D causes rickets, of which osteomalacia is the adult form[18,22]. Both diseases are marked by failure of mineralization in newly formed bone, leading to progressive demineralization and weakening of the skeleton. In children, bone growth is particularly rapid in the epiphyses and it is here that vitamin D deficiency is first manifested. In adults, on the other hand, there is slow breakdown of bone throughout the skeleton, so that the disease develops in a slower and more widespread fashion. Rickets and osteomalacia can be classified as follows[18]:

1. Rickets due to vitamin D deficiency
   (a) vitamin D deficiency in food and lack of sunlight
   (b) vitamin D deficiency in food with pigmentation of the skin
   (c) impaired absorption (idiopathic steatorrhoea, coeliac disease)
2. Vitamin D-resistant rickets
   (a) renotubular acidosis

(continued on page 464)

Structure and properties of vitamin D and related compounds

Names*	Formula and mol. wt.	Structure	Physical properties	Occurrence	Antirachitic activity
Ergosterol	$C_{28}H_{44}O$ 396.66		M.p. 168 °C (with 1½ H_2O)	Yeast, ergot, hens' eggs	None (provitamin D_2)
7-Dehydrocholesterol	$C_{27}H_{44}O$ 384.65		M.p. 150 °C (anhydrous)	Higher animals, man	None (provitamin D_3)
22-Dihydroergosterol	$C_{28}H_{46}O$ 398.68		M.p. 152 °C	Synthetic	None (provitamin D_4)
Vitamin D_2 (ergocalciferol*)	$C_{28}H_{44}O$ 396.66		M.p. 115–118 °C	Formed by irradiation of ergosterol	In rats similar to, in chicks and apes less than that of vitamin D_3
Vitamin D_3 (cholecalciferol*)	$C_{27}H_{44}O$ 384.65		M.p. 84–85 °C	Formed by irradiation of 7-dehydrocholesterol. In fish-liver oils, egg yolk, milk	Antirachitic
Vitamin D_4	$C_{28}H_{46}O$ 398.68		M.p. 96–98 °C	Formed by irradiation of 22-dihydroergosterol	Rather less than that of vitamin D_3
Ketone 250	$C_{27}H_{46}O_2$ 418.67		M.p. 73 °C	Plants, fish-liver oils	One-tenth that of vitamin D_3

					
Vitamin D_3 (cholecalciferol*)	$C_{27}H_{44}O$ 384.65		M.p. 84–85 °C	Formed by irradiation of 7-dehydrocholesterol. In fish-liver oils, egg yolk, milk	Antirachitic
Vitamin D_4	$C_{28}H_{44}O$ 398.68		M.p. 96–98 °C	Formed by irradiation of 22-dihydroergosterol	Rather less than that of vitamin D_3
Ketone 250	$C_{27}H_{42}O_3$ 418.67		M.p. 73 °C	Plants, fish-liver oils	One-tenth that of vitamin D_3
Lumisterol	$C_{28}H_{44}O$ 396.66		M.p. 118 °C	Formed by irradiation of ergosterol	None
Tachysterol	$C_{28}H_{44}O$ 396.66		Oil, readily oxidizing in air	Formed by irradiation of ergosterol	None (hypercalcaemic effect)
Dihydrotachysterol	$C_{28}H_{46}O$ 398.68		M.p. 125–127 °C	Synthetic	400 times less active than vitamin D_3 (same hypercalcaemic effect)

* Trivial names recommended by the Commission on Biochemical Nomenclature of the International Union of Pure and Applied Chemistry and the International Union of Biochemistry [*Biochim. biophys. Acta (Amst.)*, 107, 1 (1965)].

(b) FANCONI's syndrome
(c) primary vitamin D resistance
(d) renal insufficiency

The clinical symptoms of rickets are pain in the limbs, particularly the legs, genu valgum, bending of the long bones, thickening of the synchondroses of the ribs and of the joint epiphyses, and protuberance of the forehead. Tetany is also an occasional symptom. The radiological changes consist in widening of the epiphyses combined with disorganization of the epiphyseal disk, and appearance of a cup-shaped structure in place of the normal, distinct, straight boundary between metaphysis and epiphysis. The biochemical changes observed are slight lowering of the plasma calcium level, marked lowering of the plasma phosphate level, reduced urinary calcium excretion, increased phosphate clearance, increased phosphate excretion index and a rise in the plasma concentration of alkaline phosphatase; the calcium phosphate product in the plasma is lower than normal. One of the first signs of vitamin D deficiency is an increase in the amino-acid content of the urine[23].

Primary vitamin D-resistant rickets is congenital and usually hereditary. It is characterized by lower plasma phosphate levels and an increase in the phosphate excretion index. The vitamin D activity of the serum must be 10–20 times the normal value if the calcium metabolism is to be restored to normal[24]. This form of rickets may be due to a defect of vitamin D metabolism[25,33] and excretion.

Treatment

Prophylaxis of rickets: Sun-baths or quartz lamp treatment, 400 IU vitamin D per day in the pure form or as cod-liver oil.

Treatment of rickets: Rickets and osteomalacia due to a simple deficiency of vitamin D respond to daily oral doses of 3000 IU vitamin D[19]; in premature infants and children with impaired absorption these should be given intramuscularly. Treatment with massive doses has the disadvantage that there is uncertainty as to the extent to which single high doses are absorbed. High vitamin D doses are called for in primary vitamin D-resistant rickets, and treatment should begin with 50 000 IU per day[26]. Complete disappearance of the radiological and biochemical symptoms has been reported with a total of 5–400 million IU, depending on the individual[24]. The maintenance dose, again depending on the individual, ranges from 1000 to 500 000 IU per day[24]. If growth of the long bones is to proceed normally, treatment must be started immediately after birth[27].

Treatment of lupus vulgaris with vitamin D in very massive dosage is now usual only if other tuberculostatic drugs cannot be given.

Toxicology

All the D vitamins are toxic in large quantities. High doses of vitamin D mobilize the bound calcium of the skeleton and bring about a considerable increase in the plasma calcium level as well as in the urinary excretion of phosphate and calcium. The calcium mobilized from the bones is taken up in the soft tissues, particularly the kidneys and media of the vessels. The clinical symptoms are loss of appetite, gastro-intestinal disturbances, pain in the head and joints and muscular weakness; in children other signs are a dry, loose skin, tremor of the limbs, loss of muscle tone with fibrillary spasms and arterial hypertension. When death occurs, this is usually due to renal failure. The symptoms of vitamin D poisoning are reversible when ingestion of the vitamin is stopped.

The toxic effect of vitamin D appears when daily doses exceed 1000–3000 IU per kg body weight and when these doses are given over several months; in infants, hypercalcaemia may appear even with total daily doses of 3000–4000 IU[21]. The clinical appearance of this idiopathic hypercalcaemia of infancy is very similar to that of vitamin D poisoning[28]. For this reason, infants and pregnant women (since idiopathic hypercalcaemia may already occur in utero) should not be given more than 400 IU vitamin D per day[21,29]. Since enrichment of milk, margarine and baby foods with vitamin D is now quite common in many countries, the intake of this substance by infants and children is often excessive, for instance in the USA and Canada up to 2000 IU per day[21], in England up to 1200 IU per day[30].

Vitamin D_2 is less suitable for treating rickets since its hypercalcaemic effect at high dosage is greater than that of vitamin D_3. Although dihydrotachysterol has only a very slight antirachitic activity it is, like vitamin D, capable of increasing the serum calcium level. The danger of poisoning is the same, however, with both substances. In the treatment of hypercalcaemia it is essential that the serum calcium level should be watched.

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Vitamin E[1-3] (for references see page 467)

Chemistry[4]

The tocopherols are viscous, yellowish oils readily soluble in organic solvents and insoluble in water. They are stable to acids, alkalis and heat, unstable to oxidizing agents, particularly when exposed to light. The ubiquinones and ubichromenols form yellow crystals. For structure and properties of vitamin E and related compounds see the table on pages 465–466.

Assay

Biological. Antisterility test on female rats[7]; dialuric acid haemolysis test on rats[8].

Chemical[9]. Using the reducing properties of the tocopherols, for instance reduction of ferric chloride (EMMERIE-ENGEL reaction). When biological materials require to be assayed, prior separation of other reducing substances is necessary, for instance by molecular distillation or column chromatography. The individual tocopherols can be separated by column, thin-layer, paper or gas chromatography.

Unit

By weight. Formerly 1 International Unit (IU) = 1 mg *dl*-α-tocopherol acetate = 1 International Rat Unit = the amount required to be given orally to tocopherol-deficient rats to prevent absorption of the foetus in 50%[10].

1 IU = 1.00 mg *dl*-α-tocopherol acetate = 1.10 mg *dl*-α-tocopherol = 0.73 mg *d*-α-tocopherol acetate = 0.81 mg *d*-α-tocopherol.

Biogenesis

In young plants most of the vitamin E synthesized consists of α-tocopherol, whereas in seeds the other tocopherols predominate[11]. The individual steps of biogenesis are unknown but it is possible

(continued on page 466)

Structure and properties of vitamin E and r

Names*	Formula and mol. wt.	
		
Tocol	$C_{28}H_{44}O_2$ 388.64	R_1
8-Methyltocol (δ-tocopherol)	$C_{27}H_{46}O_2$ 402.67	R_1
5,8-Dimethyltocol (β-tocopherol)	$C_{28}H_{48}O_2$ 416.69	R_1
7,8-Dimethyltocol (γ-tocopherol)	$C_{28}H_{48}O_2$ 416.69	R_1
5,7,8-Trimethyltocol (α-tocopherol)	$C_{29}H_{50}O_2$ 430.72	R_1
		
8-Methyltocotrienol (δ-tocotrienol)	$C_{27}H_{40}O_2$ 396.62	R
5,8-Dimethyltocotrienol (ε-tocopherol, β-tocotrienol)	$C_{28}H_{42}O_2$ 410.65	R
7,8-Dimethyltocotrienol (γ-tocotrienol)	$C_{28}H_{42}O_2$ 410.65	R
5,7,8-Trimethyltocotrienol (ζ_2-tocopherol, α-tocotrienol, tocochromanol-3)	$C_{29}H_{44}O_2$ 424.67	R

*Trivial names recommended by the Com
national Union of Biochemistry [*Biochim.*
** Relative activity in antisterility test on

Structure and properties of vitamin E and related compounds

Names*	Formula and mol. wt.	Structure	Main source	Activity**
		Tocols		
Tocol	$C_{26}H_{44}O_2$ 388.64	$R_1 = H, R_2 = H, R_3 = H$	Synthetic	Inactive
8-Methyltocol (δ-tocopherol)	$C_{27}H_{46}O_2$ 402.67	$R_1 = H, R_2 = H, R_3 = CH_3$	Soybean oil	1
5,8-Dimethyltocol (β-tocopherol)	$C_{28}H_{48}O_2$ 416.69	$R_1 = CH_3, R_2 = H, R_3 = CH_3$	Wheat-germ oil	33
7,8-Dimethyltocol (γ-tocopherol)	$C_{28}H_{48}O_2$ 416.69	$R_1 = H, R_2 = CH_3, R_3 = CH_3$	Maize-germ oil	10
5,7,8-Trimethyltocol (α-tocopherol)	$C_{29}H_{50}O_2$ 430.72	$R_1 = CH_3, R_2 = CH_3, R_3 = CH_3$	Maize-germ oil, wheat-germ oil, etc., animal tissues	100
		Tocotrienols		
8-Methyltocotrienol (δ-tocotrienol)	$C_{27}H_{40}O_2$ 396.62	$R_1 = H, R_2 = H, R_3 = CH_3$	Palm oil	
5,8-Dimethyltocotrienol (ε-tocopherol, β-tocotrienol)	$C_{28}H_{42}O_2$ 410.65	$R_1 = CH_3, R_2 = H, R_3 = CH_3$	Wheat	5
7,8-Dimethyltocotrienol (γ-tocotrienol)	$C_{28}H_{42}O_2$ 410.65	$R_1 = H, R_2 = CH_3, R_3 = CH_3$	Rice	
5,7,8-Trimethyltocotrienol (ζ_1-tocopherol, α-tocotrienol, tocochromanol-3)	$C_{29}H_{44}O_2$ 424.67	$R_1 = CH_3, R_2 = CH_3, R_3 = CH_3$	Wheat	30

* Trivial names recommended by the Commission on Biochemical Nomenclature of the International Union of Pure and Applied Chemistry and the International Union of Biochemistry [*Biochim. biophys. Acta (Amst.)*, 107, 1 and 5 (1965)].
** Relative activity in antisterility test on rats.

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see page 467)

ous, yellowish oils readily soluble in ile in water. They are stable to acids, oxidizing agents, particularly when ones and ubichromenols form yellow operties of vitamin E and related com-s 465–466.

on female rats[7]; dialuric acid haemo-

ing properties of the tocopherols, for chloride (EMMERIE-ENGEL reaction). quire to be assayed, prior separation of necessary, for instance by molecular atography. The individual tocopherols ., thin-layer, paper or gas chromato-

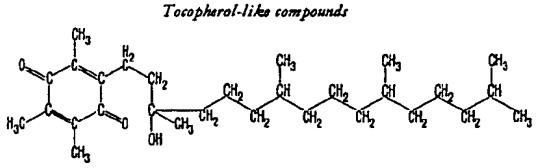
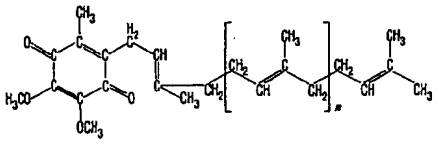
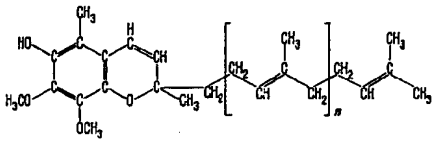
rnational Unit (IU) = 1 mg *dl*-α-toco-onal Rat Unit = the amount required ol-deficient rats to prevent absorption

opherol acetate = 1.10 mg *dl*-α-toco-erol acetate = 0.81 mg *d*-α-tocopherol.

e vitamin E synthesized consists of α-the other tocopherols predominate[11]. enesis are unknown but it is possible

(continued on page 466)

Structure and properties of vitamin E and related compounds (continued)

Names*	Formula and mol. wt.	Structure	Main source	Activity**
		Tocopherol-like compounds		
α-Tocopheryl-quinone (α-tocopherol-quinone)	$C_{29}H_{50}O_3$ 446.72		Oxidation product of α-tocopherol, green plants	Active[6]
Ubiquinones (coenzymes Q)		 $n = 4–8$	Ubiquinone-9 (ubiquinone-45, coenzyme Q_9)*: leaves; ubiquinone-10 (ubiquinone-50, coenzyme Q_{10})*: liver, yeast	
Ubichromenols		 $n = 5–8$	As corresponding ubiquinones	Active[6]

*Trivial names recommended by the Commission on Biochemical Nomenclature of the International Union of Pure and Applied Chemistry and the International Union of Biochemistry [*Biochim. biophys. Acta (Amst.)*, 107, 1 and 5 (1965)].
** Relative activity in antisterility test on rats.

that the process resembles the synthesis of the ubiquinones (formation of the terpenoid chain of mevalonic acid and of the aromatic ring from phenylalanine)[12].

Intake and excretion

The daily intake of adults in the USA has been estimated at 24 mg total tocopherols and 14 mg α-tocopherol[13], by another authority at 7.4 mg α-tocopherol[14]. The tocopherol esters are hydrolysed in the small intestine, and bile is necessary for their absorption. Probably only about 35% of the tocopherols in food is absorbed, the remainder being excreted in the faeces[15]. The normal concentration in adult serum is ca. 10 mg/l, in newborn infants ca. 5 mg/l (see page 609). The maximum blood level is reached 4–9 hours after giving tocopherols[16,17].

α-Tocopherol is stored in the liver and fatty tissues. High concentrations occur in the pituitary, adrenals, uterus and testes[18]. In the liver, tocopherol has been found in the mitochondria and microsomes[16]. The amount of tocopherols stored by the body is several grammes[2]. In the fatty tissues this is believed to include also α-tocopherylquinone, an oxidation product of α-tocopherol[19].

The metabolites tocopheronic acid and tocopheronolactone have been isolated in the form of glucuronides (Simon metabolite)[20] from the urine of persons given large amounts of tocopherol.

Function

The tocopherols act as antioxidants in the following processes both in vitro and in vivo[21,22]:

(a) they prevent the oxidation of unsaturated fatty acids (linoleic acid) to peroxides; lipoperoxides are associated with the formation of the yellowish brown pigment in smooth muscle (ceroid pigment)
(b) they prevent the oxidation of vitamin A and the carotenes
(c) they prevent the oxidation of thiol groups, particularly in enzymes, presumably in conjunction with selenium[23].

The relationship between vitamin E and cholesterol metabolism is obscure[22]. The tocopherols may play a role in nucleic acid metabolism[24] and in erythropoiesis[25]. There may be a functional connection between the tocopherols and the ubiquinones, which are involved in electron transport[26], and the formation of adenosine triphosphate (see page 403)[27]. Vitamin E seems to have an effect on the transport and metabolism of vitamin B_{12}[28].

Requirements and deficiency symptoms

Healthy adults require 10–30 mg α-tocopherol per day[29] (see also page 494), depending on the intake of polyene fatty acids[30], 0.6 mg α-tocopherol at least being required per gramme polyene fatty acid[31]. The minimal requirement of infants is probably 0.5 mg per kg body weight[32], an amount normally absorbed with the breast milk.

Vegetable oils are rich in tocopherols, especially wheat-germ oil with 200–300 mg/100 g. Other good sources of tocopherols are cereals and eggs. Animal tissues contain little tocopherol, mainly as α-tocopherol.

Vitamin E deficiency results in various changes depending on the species, age and nutritional state. They include impaired reproductivity and absorption of the foetus (rats, mice, guinea-pigs), muscular dystrophy accompanied sometimes by marked creatinuria (monkeys, mice, etc.), formation of ceroid pigments (monkeys, mice, swine), increased haemolysis in vitro (rats, chickens), encephalomalacia (chickens), exudative diathesis (chickens), necrosis of the liver ('respiratory decline' in rats), renal autolysis (rats).

In human beings the signs of vitamin E deficiency are not very marked.

A measure of the vitamin E status is provided by the peroxide haemolysis test[33]. In many persons a lowered serum tocopherol level is associated with an increase of in vitro haemolysis[30]. A low serum level is common in newborn infants and particularly premature infants, and vitamin E deficiency is a possible cause of macrocytic anaemia[34] and haemolytic anaemia[35] in infants. Vitamin E deficiency may also be caused by impaired absorption of fats[3,36]. Thus low tocopherol serum levels, often combined with creatinuria and the deposition of ceroid pigments in the smooth muscle (gastrointestinal tract), have been observed in sprue, coeliac disease, biliary cirrhosis, pancreatitis and particularly cystic fibrosis of the pancreas. A definite relationship between muscular dystrophy and vitamin E deficiency has not been proved[37]. A ve
the fatty tissues of premature infants
neous connective tissue has been repo

Treatment[39]

Administration of tocopherols is in
dication, in disturbances of fat absorpti
sis of the pancreas), in premature infa
and in persons whose diet includes la
fatty acids. In cystic fibrosis of the pan
pherol should be given daily[17]. Inclus
of women before childbirth is considere
on the capillary resistance of the infant
appear to be toxic even in large doses.

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Vitamin K[1,2]

Chemistry

The K vitamins are soluble in fats, f
stable to light. Solubility in water is con
bisulphite and the tetrasodium salt of hyd

	Main source	Activity**
	Oxidation product of α-tocopherol, green plants	Active[6]
= 4–8	Ubiquinone-9 (ubiquinone-45, coenzyme Q_9)*: leaves; ubiquinone-10 (ubiquinone-50, coenzyme Q_{10})*: liver, yeast	
= 5–8	As corresponding ubiquinones	Active[6]

Pure and Applied Chemistry and the Inter-

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ort[26], and the formation of adenosine
)[27]. Vitamin E seems to have an effect
olism of vitamin B_{12}[28].

ncy symptoms

0–30 mg α-tocopherol per day[29] (see
on the intake of polyene fatty acids[30],
st being required per gramme polyene
quirement of infants is probably 0.5 mg
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tocopherols, especially wheat-germ oil
Other good sources of tocopherols are
ssues contain little tocopherol, mainly

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state. They include impaired reproduc-
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ied sometimes by marked creatinuria
mation of ceroid pigments (monkeys,
molysis in vitro (rats, chickens), ence-
xudative diathesis (chickens), necrosis
cline' in rats), renal autolysis (rats).
is of vitamin E deficiency are not very

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persons a lowered serum tocopherol
ncrease of in vitro haemolysis[30]. A low
ewborn infants and particularly prema-
deficiency is a possible cause of macro-
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levels, often combined with creatinuria
pigments in the smooth muscle (gastro-
bserved in sprue, coeliac disease, biliary
rticularly cystic fibrosis of the pancreas.
een muscular dystrophy and vitamin E

deficiency has not been proved[37]. A very low vitamin E content in the fatty tissues of premature infants with oedema of the subcutaneous connective tissue has been reported[38].

Treatment[39]

Administration of tocopherols is indicated in intermittent claudication, in disturbances of fat absorption (particularly cystic fibrosis of the pancreas), in premature infants nurtured on cow's milk and in persons whose diet includes large amounts of unsaturated fatty acids. In cystic fibrosis of the pancreas at least 100 mg α-tocopherol should be given daily[17]. Inclusion of vitamin E in the diet of women before childbirth is considered to have a favourable effect on the capillary resistance of the infant[40]. The tocopherols do not appear to be toxic even in large doses.

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Vitamin K[1,2]

Chemistry

The K vitamins are soluble in fats, fairly stable to heat but unstable to light. Solubility in water is confined to menadione sodium bisulphite and the tetrasodium salt of hydrovitamin K_1 diphosphate.

For structure and properties of vitamin K and related compounds see the table on page 468.

Assay

Biological. By curative effect on vitamin K-deficient chicks[2].

Chemical[3]. In pure solution spectrophotometrically, fluorimetrically, colorimetrically, polarographically, also oxidimetrically after reduction to the hydroquinone.

Assay of the vitamin in animal or vegetable material must be done chromatographically or after a suitable extraction process.

Unit

No international unit; by weight. The following units are occasionally used: 20 Dam units = 1 Ansbacher unit = 0.0008 mg menadione = the minimum amount required to normalize the prothrombin time within 6 hours in vitamin K-deficient chicks weighing 70–100 g.

Biogenesis

Vitamin K_1 is synthesized in green plants under the effect of light and accumulates mainly in the chloroplasts. The K_2 vitamins are synthesized by certain intestinal bacteria. It is not known how the naphthoquinone ring is formed.

Intake and excretion

Bile or bile acids are necessary for optimal absorption of vitamin K, both from foods and from the vitamin synthesized by the intestinal flora; menadione and the synthetic water-soluble preparations are absorbed without the intervention of bile. Vitamin K is transported by the lymph. According to Martius[4], vitamin K of vegetable or bacterial origin undergoes a conversion in the animal organism in which the various side chains are replaced uniformly by an isoprenoid chain of 20 C-atoms. The original side chains are split off by the action of intestinal bacteria with the formation of methylnaphthoquinone. The latter is absorbed and converted in animal tissues into the specific vitamin $K_{2(20)}$ of the animal organism by introduction of the geranylgeranyl chain.

Vitamin K is not stored in the tissues when ingested in quantities comparable with those in the diet. When large doses are given, the vitamin accumulates in the liver and spleen[5]. After administration of radioactive vitamin K to rats, part of the activity has been found in the urine and bile[6]. Metabolites of the vitamin have been isolated from the bile of dogs[7].

Function

Vitamin K is involved in the blood coagulation mechanism and is responsible for the maintenance of a normal prothrombin time. It also affects the formation of prothrombin (factor II), factor VII, factor IX, factor X and perhaps also factor V[8]. Here the vitamin possibly acts by promoting the formation of the quaternary protein structure (S-S bridges)[9]; it may also be involved directly in prothrombin synthesis[10]. Whether the vitamin plays a role in oxidative phosphorylation is not yet clear[11], although such a role has been demonstrated in bacteria[12]. In the light of the quinone-hydroquinone structure of vitamin K and its ready conversion into a chromanol, the function of the vitamin is most likely that of an electron carrier[9].

Dicumarol and related components are vitamin K antagonists in blood coagulation, whence their use as anticoagulants; their anticoagulant effect can be inhibited by vitamin K.

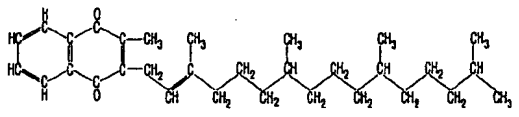
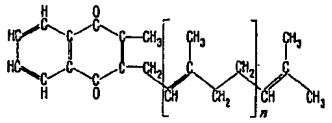
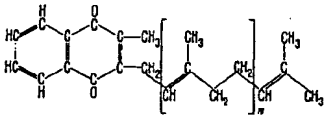
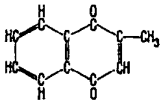
Requirements and deficiency symptoms

The human requirement of vitamin K is unknown, but ample amounts appear to be available, except in newborn infants, from a normal diet combined with synthesis by the intestinal bacteria, though the latter does not seem to play any great role in man[13]. Tests have shown that in newborn infants 5 μg disodium 2-methyl-1,4-naphthohydroquinone disuccinate per day are adequate to ensure maximum prothrombin activity[14].

Vitamin K is found mainly in green vegetables such as spinach and cabbage; the amounts in tomatoes and liver are less, and very little is present in fruits, milk and meat. Cow's milk contains more of the vitamin than breast milk.

Vitamin K deficiency causes a hypoprothrombinaemia marked by an excessive prothrombin time and a tendency to bleeding. A reduced prothrombin activity due to vitamin K deficiency occurs

Structure and properties of vitamin K and related compounds

Names*	Formula and mol. wt.	Structure	Physical properties	Occurrence	Activity **
Vitamin $K_{1(20)}$ (phyllo-quinone*)	$C_{31}H_{46}O_2$ 450.71		Yellow oil M.p. −20 °C	Green plants, tomatoes, some bacteria. Isolated from alfalfa	100
Vitamin $K_{2(20)}$ (mena-quinone-6*)	$C_{41}H_{56}O_2$ 580.90	 $n = 5$	Yellow crystals M.p. 50 °C	Isolated from putrid fishmeal	100
Vitamin $K_{2(35)}$ (mena-quinone-7*)	$C_{46}H_{64}O_2$ 649.02	 $n = 6$	Yellow crystals M.p. 54 °C	Some bacteria. Isolated from putrid fishmeal	70
Menadione (vitamin K_3, methyl-naphtho-quinone)	$C_{11}H_8O_2$ 172.19		Yellow needles M.p. 106 °C	Synthetic. Possibly a metabolite	ca. 100

* Trivial names recommended by the Commission on Biochemical Nomenclature of the International Union of Pure and Applied Chemistry and the International Union of Biochemistry [*Biochim. biophys. Acta (Amst.)*, 107, 5 (1965)].
** Relative activity in vitamin K-deficient chicks.

in some intestinal complaints like severe diarrhoea[15] and steatorrhoea[16], as well as in impaired absorption of the vitamin due to lack of bile (biliary fistula, obstruction of the bile ducts). The hypoprothrombinaemia associated with severe injury to the liver parenchyma is not due, however, to vitamin K deficiency and is also not reversed by administration of the vitamin; this forms the basis of a test of liver function. Vitamin K deficiency can also occur during long-term treatment with antibiotics or sulphonamides as a result of destruction of the intestinal flora.

During the first days of life the prothrombin activity of the plasma is 10–50% of that in adults[17], possibly because the intestinal flora is not sufficiently developed and intake of the vitamin with milk is small. Among newborn infants not given vitamin K, 0.1–1% suffer from bleeding, a proportion that has been shown to be reducible by vitamin K treatment[18,19].

Treatment

Vitamin K is given prophylactically to newborn infants, particularly premature infants and those with anoxia, the dosage of the vitamin (or of a water-soluble preparation) being 0.5–1 mg subcutaneously or intramuscularly or 1–2 mg orally at birth[18]. Double these doses may be necessary in children whose mothers have been treated with anticoagulants. Administration of the vitamin to mothers ante partum is not recommended[20]. The prophylactic use of the water-soluble menadione derivatives in pregnant women and newborn children is inadvisable on account of the danger of hyperbilirubinaemia and an increased tendency to kernicterus; these effects may be due not to the menadione derivatives themselves but to their intermediary metabolites.

The vitamin is given to correct low prothrombin activity due to a deficiency (see under 'Requirements and deficiency symptoms', above) as well as to overdosage of anticoagulants.

If possible, vitamin K should be given by the oral, intramuscular or subcutaneous route and not intravenously[21].

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Thiamine[1–3]

Structure and properties of thiamine and r

Compound	Formula and mol. wt.
Thiamine (vitamin B_1, aneurin)	$C_{12}H_{17}N_4OS$ (cation) 265.36 $C_{12}H_{18}N_4OSCl_2$ (hydrochloride) 337.27
Thiamine diphosphate (TDP) Thiamine pyrophosphate (TPP) (cocarboxylase)	$C_{12}H_{18}N_4O_7P_2S$ 424.31
α-Hydroxyethyl-2-thiamine diphosphate α-Hydroxyethyl-2-thiamine pyrophosphate	$C_{14}H_{22}N_4O_8P_2S$ 468.37
Thiochrome	$C_{12}H_{14}N_4OS$ 262.34
Oxythiamine	$C_{12}H_{14}N_3O_2S$ 266.34

Assay

Biological. Rat protection test, rat gr
Microbiological. Thiamine with *Ochro monas danica*[6]; pyrimidine and thiazol *cerevisiae*[7].
Enzymatic. With apodecarboxylase f
Chemical[9]. Quantitatively in pure chloride, gravimetrically as reineckate, derivatives from thiamine and diazo with cyanogen bromide; in biological mine to thiochrome, which shows stro violet light; with suitable modification be used to determine the mono-, di- a as well as the protein-bound thiamine.

	Physical properties	Occurrence	Activity **
	Yellow oil M.p. −20 °C	Green plants, tomatoes, some bacteria. Isolated from alfalfa	100
	Yellow crystals M.p. 50 °C	Isolated from putrid fishmeal	100
	Yellow crystals M.p. 54 °C	Some bacteria. Isolated from putrid fishmeal	70
	Yellow needles M.p. 106 °C	Synthetic. Possibly a metabolite	ca. 100

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hould be given by the oral, intramuscular
d not intravenously[21].

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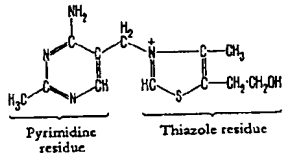
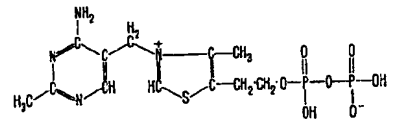
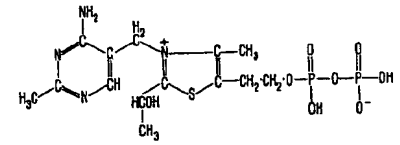
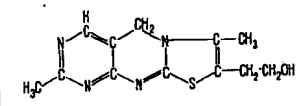
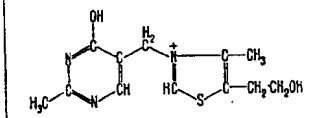
Thiamine[1-3]

Structure and properties of thiamine and related compounds

Compound	Formula and mol. wt.	Structure	Physical properties	Occurrence and activity
Thiamine (vitamin B_1, aneurin)	$C_{12}H_{17}N_4OS$ (cation) 265.36 $C_{12}H_{18}N_4OSCl_2$ (hydrochloride) 337.27		Colourless needles, readily soluble in water, odourless when pure, thermolabile in neutral and alkaline solution, stable to atmospheric oxygen, unstable to oxidizing agents and ultraviolet light M.p. 245–248 °C (hydrochloride)	Plants. In animal tissues as thiamine pyrophosphate
Thiamine diphosphate (TDP) Thiamine pyrophosphate (TPP) (cocarboxylase)	$C_{12}H_{18}N_4O_7P_2S$ 424.31		Pale yellow needles, readily soluble in water M.p. 242–244 °C	Animal tissues. Cofactor of decarboxylases and other enzymes
α-Hydroxyethyl-2-thiamine diphosphate α-Hydroxyethyl-2-thiamine pyrophosphate	$C_{14}H_{22}N_4O_8P_2S$ 468.37			Micro-organisms; represents 60% of the total thiamine in *E. coli*[4]. Also known as 'active acetaldehyde' (see page 391)
Thiochrome	$C_{12}H_{14}N_4OS$ 262.34		Yellow prisms showing blue fluorescence in solution M.p. 277–278 °C	Oxidation product of thiamine
Oxythiamine	$C_{12}H_{18}N_4O_4S$ 266.34		M.p. 195–200 °C	Antagonist of thiamine

Assay

Biological. Rat protection test, rat growth test; now little used.

Microbiological. Thiamine with *Ochromonas malhamensis*[5] or *Ochromonas danica*[6]; pyrimidine and thiazole residues with *Saccharomyces cerevisiae*[7].

Enzymatic. With apodecarboxylase from yeast[8].

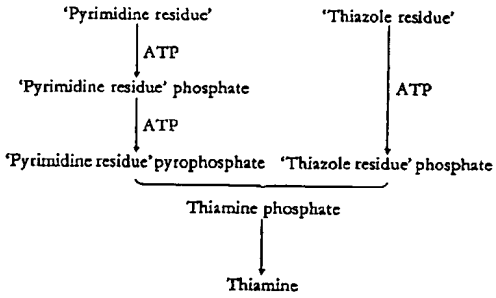
Chemical[9]. Quantitatively in pure solution by titration of the chloride, gravimetrically as reineckate, colorimetrically via the azo derivatives from thiamine and diazonium salts, fluorimetrically with cyanogen bromide; in biological material by oxidation of thiamine to thiochrome, which shows strong blue fluorescence in ultraviolet light; with suitable modifications the thiochrome method can be used to determine the mono-, di- and triphosphates of thiamine as well as the protein-bound thiamine.

Units

No international unit; by weight. The former International Unit (= 0.003 mg thiamine hydrochloride = the U.S. Pharmacopeia Unit) is now obsolete.

Biogenesis[10]

Thiamine is synthesized by plants, many bacteria and algae, and by some fungi. Some micro-organisms can synthesize only the pyrimidine and/or thiazole residues, which are formed independently of one another in a manner largely unknown and then combine to form thiamine. The first product is thiamine monophosphate, which is then hydrolysed to thiamine.



Thiamine, but not thiamine phosphate, is converted by ATP into thiamine pyrophosphate (for instance in yeast and intestinal tissue). ATP can also convert thiamine pyrophosphate into thiamine triphosphate (yeast). On the activation of aldehydes by thiamine pyrophosphate see under 'Function', below.

Intake and excretion

In the USA, the daily diet contains about 2.15 mg thiamine[11], in Germany about 1.8 mg[12]. Thiamine is readily absorbed in the small intestine and converted enzymatically in the intestinal mucosa into thiamine pyrophosphate. In rats, thiamine is synthesized by the intestinal flora[13], but in man it is unlikely that bacterial synthesis plays an important role.

In whole blood the thiamine content is 20–75 µg/l, in serum 18–62 µg/l, in spinal fluid 3–12 µg/l[16]. Erythrocytes contain 80 µg/l, leucocytes 675 µg/l[14]. The thiamine content of the serum in newborn children is very high (see page 609). Small amounts of free thiamine are present in the serum, whereas in the erythrocytes and tissues the main component is thiamine pyrophosphate. The presence of thiamine monophosphate and triphosphate and thiochrome in the tissues has often been reported[15]. The heart muscle is fairly rich in thiamine (2–3 µg/g), as are the brain, liver and kidneys (1 µg/g); smaller quantities are present in the skeletal muscles (0.5 µg/g)[16]. The human liver contains about 4 mg of thiamine[17].

When the daily dietary intake of thiamine rises above 0.5–0.6 mg the urinary excretion of the vitamin increases in proportion to the intake; on an ample diet it amounts to at least 100 µg/day[2,18] (see also page 676). The urine also contains breakdown products (pyrimidine and thiazole residues) the amounts of which are not proportional to the thiamine content of the diet, so that they are regarded as a measure of the rate at which body stores of thiamine are being depleted[19]. The thiamine content of breast milk (see page 689) depends on the thiamine intake and shows large individual variations.

Function

Thiamine pyrophosphate possesses coenzyme functions in the breakdown of carbohydrates (oxidative decarboxylation of pyruvate, see page 391), in the citric acid cycle (oxidative decarboxylation of α-ketoglutarate, see page 390), in the pentose phosphate cycle (transketolase reaction, see page 421) and other biochemical reactions[20]; at least 24 enzymes are known that contain thiamine pyrophosphate as coenzyme[2]. The active aldehyde group in these reactions is formed or transported by thiamine pyrophosphate enzymes and is bound to the C-2 atom of the thiazole ring (see table).

Reaction	Active aldehyde
Oxidative decarboxylation of pyruvate	Active pyruvate (α-lactyl-2-thiamine pyrophosphate)[21]
	Active acetaldehyde (α-hydroxyethyl-2-thiamine pyrophosphate)[21]
Oxidative decarboxylation of α-ketoglutarate	Active α-ketoglutarate (?)
	Active succinate semialdehyde (?)
Transketolase reaction	Active xylulose 5-phosphate (?)
	Active glycolaldehyde[21]
	Active sedoheptulose 7-phosphate (?)
Glyoxylate carboligase reaction	Active glyoxylate[22]
	Active formaldehyde[22]
	Active tartronic semialdehyde[22]

Thiamine pyrophosphate plays an important part in the production of stimuli in the peripheral nerves and in the recovery process after stimulation[23]; during stimulation of the peripheral nerves thiamine is liberated from thiamine pyrophosphate[24].

The thiamine-sparing effect of dietary fats probably derives from the fact that in thiamine deficiency the activity of pyruvate dehydrogenase is more rapidly inhibited than that of oxoglutarate dehydrogenase (α-ketoglutarate dehydrogenase)[2]; in thiamine deficiency, however, toxic products may also be formed from carbohydrates[20].

Various synthetic compounds resembling thiamine in structure, such as oxythiamine, pyrithiamine and neopyrithiamine, act as thiamine antagonists[25]; antithiamine factors of unknown structure occur in bacteria, plants and animals (particularly in cold-blooded animals, where the antimetabolite concerned is also known as 'thiaminase')[26].

Requirements and deficiency symptoms

The requirement of thiamine depends primarily on the intake of carbohydrates, although in practice it is usually related to the calorie intake. In adults the minimum requirement is about 0.27–0.33 mg per 1000 kcal[28]. In bottle-fed infants the maintenance dose has been given as 0.14–0.20 mg per day[29]. The Joint FAO/WHO Expert Group[27] recommends a daily thiamine intake of 0.4 mg per 1000 kcal for infants, children, adults and pregnant and lactating women (see page 493). The allowances of the Food and Nutrition Board (USA)[33] (see page 494) are based on a daily intake of 0.5 mg thiamine per 1000 kcal. The requirement of thiamine increases with the metabolic rate. The possible presence of antithiamine factors in the diet must also be allowed for.

Good sources of thiamine are yeast, pork, liver, kidneys and wholemeal cereals (see pages 507–508). The vitamin is partly destroyed during cooking, particularly in alkaline media, but is unaffected by deep freezing[1].

The classical symptoms of vitamin B_1 deficiency are incipient anorexia, nausea and vomiting. Other symptoms are fatigue, weakness, hypotonia of the gastrointestinal tract and disturbances of the peripheral nerves (weakness in the limbs, hyperaesthesia and paraesthesia, disturbances of coordination). Emotional disturbances are also observed, such as depression, irritability and impairment of memory and power of concentration.

Beriberi takes various forms according to the predominating symptoms:

(a) An exudative form, in which oedema is the first symptom. This may be followed by enlargement of the heart and right-sided heart failure with sudden death.

(b) A 'dry' form in which the main symptoms are polyneuritis of peripheral degenerative type and atrophy of the muscles of the limbs. In European latitudes thiamine deficiency is marked mainly by polyneuritis; it is seen for example in chronic alcoholism, although this condition is probably accompanied in general by deficiency of several of the B vitamins[30].

(c) A rare cerebral form with the symptoms of WERNICKE's disease, namely nystagmus, ocular paralysis and emotional disturbances (irritability, sleeplessness, loss of memory, disorientation, confabulation, hallucinations), followed by loss of consciousness and death. This disease is seen for instance in Europe among chronic alcoholics and occasionally in patients with cancer.

(d) An infantile form seen in the first year of life and a principal cause of the high infant mortality in southern and southeastern Asia; thus between 1954 and 1958 in the Philippines 15 000 children died each year from beriberi[31]. The chronic form is manifested by a slow growth rate, constipation, vomiting and oedema, the acute form by heart failure and death. Occasionally the symptoms resemble those of meningitis or encephalitis. The cause of this vitamin deficiency disease is still to some extent obscure. In most cases the mother suffers from thiamine deficiency so that the maternal milk is deficient in this vitamin; toxic substances in the milk may also play a role[32].

Biochemically, thiamine deficiency is characterized by a low urinary concentration of the vitamin (in beriberi 0–14 µg per 24 h), by disturbances of carbohydrate metabolism (increased blood pyruvate and α-ketoglutarate levels[33]) and by a low tissue concentration of thiamine pyrophosphate (erythrocytes[34], brain[35]). The thiamine pyrophosphate content of the erythrocytes can be measured by means of the transketolase activity, which in thiamine deficiency can be normalized by administering thiamine pyrophosphate[34]. The biochemical changes precede the pathological symptoms; it is not known, however, how the lesio the heart and in the nervous system aris to the tissues is too low, or there may metabolites.

Order in which thiamine deficiency sympto per day)[34]

Days	Biochemical symptoms
5	Urinary thiamine 50 µg/24 h
10	Urinary thiamine 25 µg/24 h, transketolase activity of erythrocytes somewhat low
21–28	Urinary thiamine 0–25 µg/24 h, transketolase activity of erythrocytes 15–25% lower
30–200	Urinary thiamine negligible, transketolase activity of erythrocytes 35% lower
over 200	Urinary thiamine negligible, transketolase activity of erythrocytes over 45% lower

Treatment

In adults beriberi is usually treated b a dosage of 20–30 mg thiamine per d usually successful, but when the heart is should be given initially by the paren renteral administration results in an chemical changes usually regress rapi turbances are normally reversible but is slow and there is occasional irreve Thiamine has been used in the treatm a condition of its successful application deficiency.

Since thiamine hydrochloride is only ily decomposed in alkaline media, bi with fewer disadvantages[36], for inst phide, have been synthesized.

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is not known, however, how the lesions in the vascular system of the heart and in the nervous system arise. Possibly the energy supply to the tissues is too low, or there may be an accumulation of toxic metabolites.

Order in which thiamine deficiency symptoms appear (0.2 mg thiamine per day)[34]

Days	Biochemical symptoms	Clinical symptoms
5	Urinary thiamine 50 µg/24 h	–
10	Urinary thiamine 25 µg/24 h, transketolase activity of erythrocytes somewhat low	–
21–28	Urinary thiamine 0–25 µg/24 h, transketolase activity of erythrocytes 15–25% lower	Loss of weight, sleeplessness, irritability
30–200	Urinary thiamine negligible, transketolase activity of erythrocytes 35% lower	Increasing weakness, loss of weight, polyneuritis, bradycardia, peripheral oedema, enlargement of heart, ophthalmoplegia
over 200	Urinary thiamine negligible, transketolase activity of erythrocytes over 45% lower	Histopathological changes as a result of the biochemical defects

Treatment

In adults beriberi is usually treated by administering thiamine at a dosage of 20–30 mg thiamine per day[36]. Oral administration is usually successful, but when the heart is severely affected the vitamin should be given initially by the parenteral route. In rare cases parenteral administration results in anaphylactic shock. The biochemical changes usually regress rapidly; the cardiovascular disturbances are normally reversible but recovery from polyneuritis is slow and there is occasional irreversible injury to the nerves. Thiamine has been used in the treatment of many diseases[37], but a condition of its successful application is the existence of a thiamine deficiency.

Since thiamine hydrochloride is only partly absorbed and is readily decomposed in alkaline media, biologically active derivatives with fewer disadvantages[38], for instance thiamine propyl disulphide, have been synthesized.

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Riboflavin[1–3] (for references see page 473)

Chemistry

For structure and properties of riboflavin and related compounds see the table on page 472.

Assay

Biological[5]. Growth test on rats or chicks.

Microbiological[6]. With *Lactobacillus helveticus* (*L. casei*), *Leuconostoc mesenteroides* or *Tetrahymena pyriformis*[7].

Chemical[4]. By mass analysis after oxidation with periodate; polarographically, spectrophotometrically in the UV range; by photometric measurement of the colour; by fluorometric measurement of the colour of the original vitamin or after conversion to lumiflavin by irradiation in alkaline solution. In biological materials assay is best carried out by the latter method after suitable pre-treatment. The individual flavins can be separated by paper chromatography, electrophoresis or ion exchange.

Unit

No international unit; by weight. 1 rat unit = 4 µg riboflavin.

Biogenesis[8]

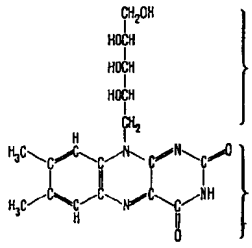
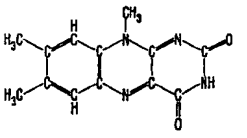
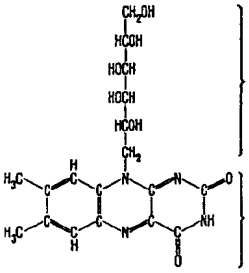
Riboflavin is synthesized by bacteria (such as *Clostridium* spp., *Azotobacter* spp.), by fungi (such as ascomycetes and yeasts) and by plants. Purines, pyrimidines, riboflavin and pteridines are formed in a similar way from glycine, formic acid and carbon dioxide. Probable intermediate products of the biosynthesis are 4,5-diaminouracil and 6,7-dimethyl-8-ribityl-lumazine, so that the pyrimidine ring is the first product. The benzene ring is probably completed by the incorporation of acetate. Riboflavin is possibly synthesized at the FAD stage, with 4,5-diaminouracil adenine dinucleotide as intermediate. The conversion of riboflavin into flavin mononucleotide and flavin adenine dinucleotide is mediated by ATP and also occurs in animal tissues (intestine, liver).

Intake and excretion

In USA the daily diet contains on the average 2.65 mg riboflavin[9]. Riboflavin is absorbed in the small intestine in amounts proportional to the amounts of the vitamin ingested[10]. Riboflavin is also synthesized by the intestinal flora, particularly when the diet contains large amounts of carbohydrates that are not easily digested. A part of the riboflavin so formed may also be absorbed in man[11]. In rats, FAD is absorbed less easily than free riboflavin and FMN[12]. Free riboflavin is converted in the intestinal mucosa into FMN, which is then transformed into FAD in the liver[13].

The riboflavin content of whole blood[14] is 49–104 µg/l, of serum[15] 26–37 µg/l, of erythrocytes[16] 180–262 µg/l, of leucocytes[15] 2.27–2.93 mg/l, of liver[16] 25 µg/g, of heart muscle[16] 13 µg/g, of skeletal muscle[16] 2.7 µg/g, of the lens of the eye[16] 3.1 µg/g. In blood (see page 609) and in animal organs 70–90% of the riboflavin is present as FAD, 5–30% as FMN and 0.5–2% as free riboflavin[4]. In the presence of a positive nitrogen balance[17] flavoproteins are produced in the tissues (with decreasing urinary riboflavin concentration); in the presence of a negative nitrogen balance[17] or during fasting[18] flavoproteins are broken down in the tissues (with increasing urinary riboflavin concentration). The urinary excretion varies with the intake; when the latter is less than 1 mg riboflavin

Structure and properties of riboflavin and related compounds

Names	Formula and mol. wt.	Structure	Physical properties	Occurrence and activity
Riboflavin (vitamin B_2, lactoflavin, 7,8-dimethyl-10-[D-ribityl]-isoalloxazine)	$C_{17}H_{20}N_4O_6$ 376.37		Orange-yellow needles, bitter taste, slightly soluble in water and ethanol, readily soluble in acids, stable to heat when dry and to acid media, very unstable to alkalis and light. Yellowish green fluorescence in solution M.p. 275–282 °C (decomp.)	Constituent of flavin mononucleotide and flavin adenine dinucleotide. Occurs in free form in some micro-organisms. Makes up 0.5–2% of total riboflavin of animal organs[4]. Present in urine
Flavinmononucleotide (FMN) (riboflavin-5'-phosphate)	See page 345	See page 345	Yellow powder, soluble in water	In micro-organisms as active group of flavoproteins, and in plants and animals. Makes up 5–30% of total riboflavin of animal organs[4]
Flavinadenine dinucleotide (FAD)	See page 345	See page 345	Yellow powder, readily soluble in water, insoluble in ethanol	In micro-organisms as active group of flavoproteins, and in plants and animals. Makes up 70–90% of riboflavin of animal organs[4]
Lumiflavin (7,8,10-trimethyl-isoalloxazine)	$C_{13}H_{12}N_4O_2$ 256.27		Yellow crystals, only slightly soluble in water. Blue fluorescence in solution M.p. 333 °C (decomp.)	Formed from riboflavin by irradiation in alkaline solution. Antagonist of riboflavin
Galactoflavin (7,8-dimethyl-10-[D-dulcityl]-isoalloxazine)	$C_{18}H_{22}N_4O_7$ 406.40		Yellow crystals, slightly soluble in water	Antagonist of riboflavin

per day, 10% of the intake is excreted; with an intake of 1.5 mg, about 20% is excreted, with an intake of 5–11 mg, about 60%[17,19] (see also page 676). The concentration of riboflavin in breast milk depends on the intake of the vitamin[20].

Function

In the form of flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) riboflavin forms the active group of the flavoproteins, enzymes with an important function in biological oxidations (see pages 402–403). Here the isoalloxazine system acts as a reversible redox system. In the oxidized form (fluorescent) the flavoproteins take up two hydrogen atoms and pass into the leuco-form (non-fluorescent); in some reactions they take up only one hydrogen atom and become semiquinones. At least 40 flavoproteins are known[21], among them (active group in brackets) important oxidases such as aldehyde oxidase (FAD), xanthine oxidase (FAD), L-amino acid oxidase (FMN), D-amino acid oxidase (FAD), dehydrogenases like acyl-CoA-dehydrogenase (FAD) and succinate dehydrogenase (FAD), NAD(P)H_2 dehydrogenases (FAD), and glutathione reductase (FAD).

Riboflavin, in combination with protein, is necessary to prevent recurrent skin lesions such as those occurring in the corners of the mouth[1]. In erythropoiesis, it is possibly necessary for the formation or effective functioning of erythropoietin[22].

Requirements and deficiency symptoms

The requirement of riboflavin is usually based on the energy requirement[23] but can also be related to the protein requirement[24].

Depletion-repletion studies indicate th
flavin are met by a daily intake of 0.44 n
figure that the daily intake of 0.55 mg,
a Joint FAO/WHO Expert Group[25] i
Food and Nutrition Board (USA)[26] l
(page 494) on studies of riboflavin re
pendence of these on 'metabolic body si
power of the body weight: infants 0.
0.09 mg, children 12–14 years 0.08 mg
$kg^{0.75}$. For the requirements of pregn
the table on page 494.

Good sources of riboflavin are milk
tein and green vegetables (see pages 49
stable to cooking, but up to 85% of t
exposure to sunlight[2].

Experimental riboflavin deficiency i
of the intestinal mucosa (glossitis, i
pharyngeal mucosa, cheilitis, rhagades
of the skin (severe pruritis, desquamat
of the skin creases over joints, seborrh
larly of the anogenital region (inflamm
vere pruritis of anus, vulva and scrotu
also indicated injury to the bone marro
rocytes) with normochromic and no
ciency of reticulocytes. Whether the
and capillary dilatation of the skin see
ciency (ariboflavinosis) are due to acti
is uncertain[1,27], and these symptoms m
nutritional defect. Biochemically, ribo
cognized by the low riboflavin conter
276 µg/l at a daily intake of 2.55–3.55
intake of 0.55 mg[29]) and by the reduc
vitamin (below 40 µg/24 h[30]).

Treatment

The deficiency symptoms usually dis
giving riboflavin in doses of 5 mg 2–3
this treatment will confirm the diagnos

Vitamin B_6[1,2] (for references see page 47

Chemistry

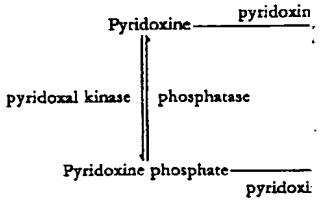
For structure and properties of vitami
see the table on page 474.

Assay[3,4]

Biological. Growth test on chicks and
deficiency dermatitis.

Microbiological. With *Saccharomyces ca*
pyridoxine, pyridoxamine and pyridc
46%, pyridoxamine 40% of activity
casei (pyridoxal activity only), *Strepto*
and pyridoxal activity only), *Tetrahymen*
and pyridoxal activity only).

Enzymatic. Pyridoxal phosphate by n
lase.



ıl properties	Occurrence and activity
yellow bitter taste, soluble in d ethanol, oluble in ıble to heat y and to acid ery unstable s and light. ih green ence in 5–282 °C .)	Constituent of flavin mononucleotide and flavin adenine dinucleotide. Occurs in free form in some micro-organisms. Makes up 0.5–2% of total riboflavin of animal organs[4]. Present in urine
powder, n water	In micro-organisms as active group of flavoproteins, and in plants and animals. Makes up 5–30% of total riboflavin of animal organs[4]
powder, oluble in soluble in	In micro-organisms as active group of flavoproteins, and in plants and animals. Makes up 70–90% of riboflavin of animal organs[4]
crystals, only soluble in lue fluores- solution) °C .)	Formed from riboflavin by irradiation in alkaline solution. Antagonist of riboflavin
crystals, soluble in	Antagonist of riboflavin

(active group in brackets) important
dase (FAD), xanthine oxidase (FAD),
), D-amino acid oxidase (FAD), de-
dehydrogenase (FAD) and succinate
D(P)H_2 dehydrogenases (FAD), and

. with protein, is necessary to prevent
those occurring in the corners of the
s possibly necessary for the formation
ythropoietin[22].

cy symptoms

avin is usually based on the energy
related to the protein requirement[24].

Depletion-repletion studies indicate that the body's needs of riboflavin are met by a daily intake of 0.44 mg/1000 kcal, and it is on this figure that the daily intake of 0.55 mg/1000 kcal recommended by a Joint FAO/WHO Expert Group[25] is based (see page 493). The Food and Nutrition Board (USA)[26] base their recommendations (page 494) on studies of riboflavin requirements that point to dependence of these on 'metabolic body size', represented as the 0.75th power of the body weight: infants 0.1 mg, children 10–12 years 0.09 mg, children 12–14 years 0.08 mg, adults 0.07 mg riboflavin/$kg^{0.75}$. For the requirements of pregnant and lactating women see the table on page 494.

Good sources of riboflavin are milk, liver, kidneys, heart, protein and green vegetables (see pages 499–515). The vitamin is fairly stable to cooking, but up to 85% of that in milk is destroyed on exposure to sunlight[2].

Experimental riboflavin deficiency in man has resulted in lesions of the intestinal mucosa (glossitis, inflammation of the buccopharyngeal mucosa, cheilitis, rhagades of the corners of the mouth), of the skin (severe pruritis, desquamation, rhagades, inflammation of the skin creases over joints, seborrhoeic dermatitis) and particularly of the anogenital region (inflammation, desquamation and severe pruritis of anus, vulva and scrotum); recent studies[27,28] have also indicated injury to the bone marrow (absence of mature erythrocytes) with normochromic and normocytic anaemia and deficiency of reticulocytes. Whether the vascularization of the cornea and capillary dilatation of the skin seen in endemic riboflavin deficiency (ariboflavinosis) are due to actual deficiency of the vitamin is uncertain[1,27], and these symptoms may be the result of a multiple nutritional defect. Biochemically, riboflavin deficiency can be recognized by the low riboflavin content in the erythrocytes (202–276 µg/l at a daily intake of 2.55–3.55 mg, 100–131 µg/l at a daily intake of 0.55 mg[28]) and by the reduced urinary excretion of the vitamin (below 40 µg/24 h[30]).

Treatment

The deficiency symptoms usually disappear after several days of giving riboflavin in doses of 5 mg 2–3 times a day[2]. The results of this treatment will confirm the diagnosis.

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Vitamin B_6[1,2] (for references see page 476)

Chemistry

For structure and properties of vitamin B_6 and related compounds see the table on page 474.

Assay[3,4]

Biological. Growth test on chicks and rats, curatively on rats with deficiency dermatitis.

Microbiological. With *Saccharomyces carlsbergensis* (same activity for pyridoxine, pyridoxamine and pyridoxal), *S. cerevisiae* (pyridoxal 46%, pyridoxamine 40% of activity of pyridoxine), *Lactobacillus casei* (pyridoxal activity only), *Streptococcus faecalis* (pyridoxamine and pyridoxal activity only), *Tetrahymena pyriformis*[5] (pyridoxamine and pyridoxal activity only).

Enzymatic. Pyridoxal phosphate by means of tyrosine decarboxylase.

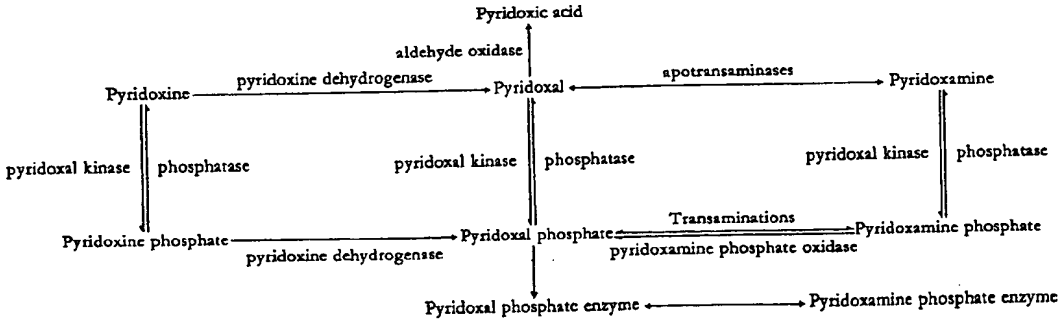
Chemical[6]. Spectrophotometrically or colorimetrically in pure solution on the basis of the reactivity of the phenolic hydroxyl group; pyridoxal and pyridoxal phosphate fluorimetrically as cyanohydrin (pyridoxine is converted into pyridoxal by oxidation, pyridoxamine to the same compound by transamination); pyridoxic acid fluorimetrically as lactone (pyridoxamine can be converted into pyridoxine; pyridoxine and pyridoxal can be oxidized to pyridoxic acid); all the above forms of the vitamin can be separated by column, paper or thin-layer chromatography.

Unit

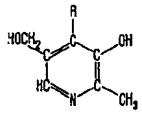
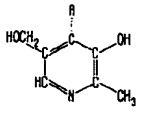
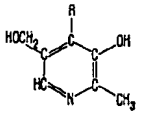
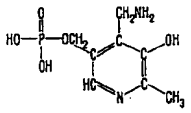
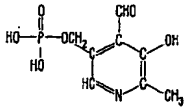
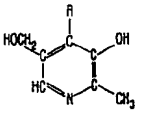
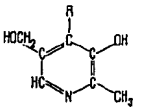
No international unit; by weight.

Biogenesis[7]

Practically nothing is known of the biosynthesis of the pyridine ring. Pyridoxine, pyridoxal, pyridoxamine and the phosphates are interconvertible in animal tissues and by micro-organisms in accordance with the scheme shown[8].



Structure and properties of vitamin B_6 and related compounds

Names*	Formula and mol. wt.	Structure	Physical properties	Occurrence and activity
Pyridoxine*, pyridoxol* (adermine)	$C_8H_{11}NO_3$ 169.18	 R = $-CH_2OH$	Colourless crystals, water-soluble, stable to heat, unstable to light M. p. 160 °C	Particularly in plants. Vitamin B_6 activity for higher plants and yeasts; only slight activity for bacteria
Pyridoxamine*	$C_8H_{12}N_2O_2$ 168.20	 R = $-CH_2NH_2$	Colourless crystals, water-soluble, unstable to heat and light M. p. 193 °C	Particularly in animal tissues. Vitamin B_6 activity for micro-organisms and higher animals
Pyridoxal*	$C_8H_9NO_3$ 167.17	 R = $-CHO$	Colourless crystals, water-soluble, unstable to heat and light	Particularly in animal tissues. Vitamin B_6 activity for micro-organisms and higher animals
Pyridoxamine phosphate	$C_8H_{13}N_2O_5P$ 248.18		–	Coenzyme in transaminations
Pyridoxal phosphate (codecarboxylase)	$C_8H_{10}NO_6P$ 247.15		Yellow crystals, water-soluble M. p. > 270 °C	Particularly in muscle. Coenzyme in decarboxylations, transaminations and phosphorylations
Pyridoxic acid (4-pyridoxic acid)	$C_8H_9NO_4$ 183.17	 R = $-COOH$	White crystals, moderately soluble in water M. p. 247 °C	Particularly in urine (breakdown product). No vitamin B_6 activity
Deoxypyridoxine	$C_8H_{11}NO_2$ 153.18	 R = $-CH_3$	–	Vitamin B_6 antagonist in micro-organisms and animals

* Trivial names recommended by the Commission on Biochemical Nomenclature of the International Union of Pure and Applied Chemistry and the International Union of Biochemistry [*Biochim. biophys. Acta (Amst.)*, 107, 1 (1965)].

Intake and excretion

An average daily diet contains abou
B_6 phosphates from foods are probabl
by phosphatases[9], and the non-phospl
go absorption in the upper intestinal
also by the intestinal flora, though it i
makes use of this source of the vitamir
in the faeces is largely independent of
adults to 0.7–0.9 mg per day and in ci
day[2]. For urinary excretion of the vit

The non-phosphorylated compound
phates in the tissues (mainly the brain,
ably not in the blood, by the action of
and ATP[11]. The vitamin B_6 activity
20–45 µg/l, of the serum 30–80 µg/l[12]. P
the serum (ca. 10 µg/l) and leucocytes (s
doxine does not seem to be present in th
istration results within 3 days in an inc
phate content of whole blood and withi
in the leucocytes[2]. The human body ca
mum of 7 mg pyridoxine per day into
intakes of pyridoxine do not cause any
doxal phosphate content of the blood[1

Vitamin B_6 is stored in the liver (5–2
and brain (12–25 µg/g)[12], and it may b
(see page 639). The vitamin B_6 content
estimated variously at 40–150 mg[14] and
the pyridoxal phosphate in the body app
phosphorylase in the muscles[16]. The d
is 2.2–4.4%, with a 2–3% depletion o

Pyridoxal is converted in the liver b
doxic acid, which is excreted in the urir
of pyridoxine, pyridoxal and pyridoxa
normal diet about a half or less of the
as pyridoxic acid in the urine[14,17].

Function[8,18]

Vitamin B_6 is involved as coenzym
actions[19]. Pyridoxamine phosphate an
coenzymes in transamination reaction
down of γ-aminobutyric acid in the br
abolism. Pyridoxal phosphate is the co
tion of amino acids and for other rea
the table below). Pyridoxal phosphate
reactions of tryptophan metabolism (
made use of in the tryptophan loading
B_6 deficiency[20]. Pyridoxal phosphate
transport of one-carbon units from seri
and plays a role in the formation of ci
also involved with other cofactors in th
linic acid, a precursor of the porphyrins (
phosphate is a component of α-glucan
certain whether vitamin B_6 is directly in

Deoxypyridoxine and other synthet
(such as isonicotinic acid hydrazide) an
B_6 antagonists[25], with various degree
zymes linked to pyridoxal phosphate.

Enzymatic reactions with pyridoxamine pho

Enzyme	
Diaminoxidase, histaminase	Oxidatic
Serine hydroxymethyl transferase	Formati hydrofol
α-Glucan phosphorylase	Phosphc
Transaminases	amino a acid$_1$ + occurrin
Synthases	Formati and indc methylcy anethiol

* Pyridoxamine phosphate acts as cofactor

cal properties	Occurrence and activity
less crystals, soluble, stable , unstable to 60 °C	Particularly in plants. Vitamin B_6 activity for higher plants and yeasts; only slight activity for bacteria
less crystals, soluble, le to heat and 93 °C	Particularly in animal tissues. Vitamin B_6 activity for micro-organisms and higher animals
less crystals, soluble, le to heat and	Particularly in animal tissues. Vitamin B_6 activity for micro-organisms and higher animals
	Coenzyme in transaminations
v crystals, soluble > 270 °C	Particularly in muscle. Coenzyme in decarboxylations, transaminations and phosphorylations
crystals, ately soluble er 247 °C	Particularly in urine (breakdown product). No vitamin B_6 activity
	Vitamin B_6 antagonist in micro-organisms and animals

of Pure and Applied Chemistry and the Inter-

Intake and excretion

An average daily diet contains about 2 mg vitamin B_6[2]. Vitamin B_6 phosphates from foods are probably hydrolysed in the intestine by phosphatases[8], and the non-phosphorylated compounds undergo absorption in the upper intestinal tract. Vitamin B_6 is formed also by the intestinal flora, though it is hardly likely that the body makes use of this source of the vitamin[10]. Excretion of vitamin B_6 in the faeces is largely independent of the intake and amounts in adults to 0.7–0.9 mg per day and in children to 0.15–0.30 mg per day[2]. For urinary excretion of the vitamin see page 676.

The non-phosphorylated compounds are converted into the phosphates in the tissues (mainly the brain, liver and kidneys), but probably not in the blood, by the action of the enzyme pyridoxal kinase and ATP[11]. The vitamin B_6 activity of whole blood amounts to 20–45 µg/l, of the serum 30–80 µg/l[12]. Pyridoxal phosphate occurs in the serum (ca. 10 µg/l) and leucocytes (see also pages 609–610). Pyridoxine does not seem to be present in the blood[4]. Pyridoxine administration results within 3 days in an increase in the pyridoxal phosphate content of whole blood and within 10 days in a similar increase in the leucocytes[2]. The human body can apparently convert a maximum of 7 mg pyridoxine per day into pyridoxal phosphate; higher intakes of pyridoxine do not cause any further increase in the pyridoxal phosphate content of the blood[13].

Vitamin B_6 is stored in the liver (5–20 µg/g), muscles (2–6 µg/g) and brain (12–25 µg/g)[12], and it may be present in the spinal fluid (see page 639). The vitamin B_6 content of the whole body has been estimated variously at 40–150 mg[14] and 16–32 mg[15]; about a half of the pyridoxal phosphate in the body appears to be bound to α-glucan phosphorylase in the muscles[16]. The daily turnover of vitamin B_6 is 2.2–4.4%, with a 2–3% depletion of the body's reserves[14].

Pyridoxal is converted in the liver by aldehyde oxidase to pyridoxic acid, which is excreted in the urine along with small amounts of pyridoxine, pyridoxal and pyridoxamine (see page 676). On a normal diet about a half or less of the vitamin B_6 turnover appears as pyridoxic acid in the urine[14,17].

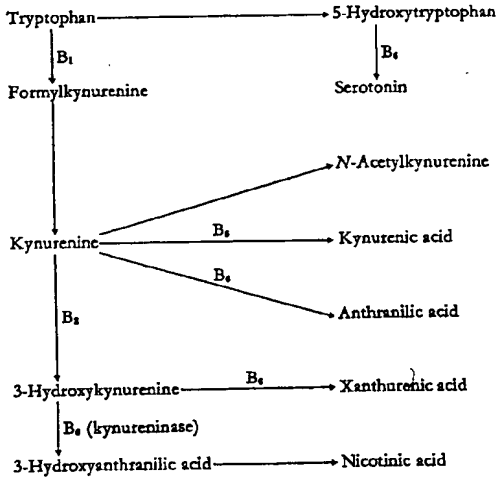
Function[8,18]

Vitamin B_6 is involved as coenzyme in over 40 enzymatic reactions[19]. Pyridoxamine phosphate and pyridoxal phosphate act as coenzymes in transamination reactions important for the breakdown of γ-aminobutyric acid in the brain and for oxalic acid metabolism. Pyridoxal phosphate is the coenzyme in the decarboxylation of amino acids and for other reactions of amino acids (see the table below). Pyridoxal phosphate is also involved in various reactions of tryptophan metabolism (see figure), and this fact is made use of in the tryptophan loading test for diagnosis of vitamin B_6 deficiency[20]. Pyridoxal phosphate is also the coenzyme in the transport of one-carbon units from serine to tetrahydrofolic acid[21], and plays a role in the formation of circulating antibodies[22]. It is also involved with other cofactors in the synthesis of δ-aminolaevulinic acid, a precursor of the porphyrins (haemopoiesis)[23]. Pyridoxal phosphate is a component of α-glucan phosphorylase[16]. It is uncertain whether vitamin B_6 is directly involved in fat metabolism[24].

Deoxypyridoxine and other synthetic pyridoxines, hydrazides (such as isonicotinic acid hydrazide) and cycloserine act as vitamin B_6 antagonists[25], with various degrees of inactivation of the enzymes linked to pyridoxal phosphate.

Dependence of tryptophan metabolism on the B vitamins[20, 26]

Bold arrows indicate the main breakdown route. In vitamin B_6 deficiency, kynureninase is inactivated more strongly than the transaminases, which are involved in the formation of xanthurenic acid[27].



Requirements and deficiency symptoms

In adults the minimum requirement of pyridoxine hydrochloride is apparently about 1.25 mg per day with a daily protein intake of 30 g and 1.5 mg per day with a daily protein intake of 100 g, the optimal requirement being 1.75–2.0 mg per day with a daily protein intake of 100 g[28, 29]. The requirement of the vitamin increases with the protein intake. In infants it is between 0.1 and 0.5 mg per day and is dependent on the protein intake (20 µg/g protein); the minimum requirement of children is 0.5–1.5 mg per day, that of adolescents 1.5–2 mg per day[34]. The Food and Nutrition Board (USA) recommend a vitamin B_6 intake of 2.0 mg per day when the daily protein intake is 100 g or more[30]. The human vitamin B_6 requirement has been the object of much discussion[31, 32]; it is possible that even higher intakes than those given above are desirable. During pregnancy the requirement is probably increased[33], and a daily intake of 2.5 mg has been recommended for pregnant and lactating women[30]. See also page 494.

Vitamin B_6 occurs in practically all vegetable and animal foodstuffs (see pages 499–515). Good sources of vitamin B_6 are yeast, liver and cereals (wholemeal, since milling results in loss of 80–90% of the vitamin)[2].

The symptoms of vitamin B_6 deficiency vary greatly with the species and age of the individual. The great variety of deficiency symptoms observed is in part due to the fact that in progressive B_6 deficiency not all the enzyme systems are blocked simultaneously to

*Enzymatic reactions with pyridoxamine phosphate and pyridoxal phosphate as cofactors**

Enzyme	Reaction	Enzyme	Reaction
Diaminoxidase, histaminase	Oxidation of diamines and histamine	Decarboxylases	For example, decarboxylation of histidine to histamine, tyrosine to tyramine, dopa to dopamine, hydroxytryptophan to serotonin
Serine hydroxymethyl transferase	Formation of 5,10-methylene tetrahydrofolic acid	Threonine aldolase	Breakdown of threonine to glycine and acetaldehyde
α-Glucan phosphorylase	Phosphorolysis of glycogen	Dehydratases	Deamination of serine, homoserine, threonine, etc.
Transaminases	amino acid$_1$ + ketoic acid$_2$ ⇌ ketoic acid$_1$ + amino acid$_2$ (with all naturally occurring amino acids)	Desulphydrases	Deaminating desulphydration of cysteine and homocysteine
Synthases	Formation of tryptophan from serine and indole, of cysteine from serine, of methylcysteine from serine and methanethiol	Racemases	L-amino acid ⇌ D-amino acid (alanine, methionine, glutamic acid)

* Pyridoxamine phosphate acts as cofactor only in transaminations.

the same extent[37]. The following symptoms have been observed in experimental B_6 deficiency[37]: in rats, severe dermatitis (rat pellagra), occasionally haemolytic anaemia and overall loss of body fat; in rabbits, desquamating dermatitis of the ears, mild anaemia, convulsions, creatinuria, paralytic collapse and death[35]; in rhesus monkeys, arteriosclerosis, dental caries, fatty degeneration or cirrhosis of the liver, pancreatic sclerosis, disturbances of the central nervous system[38]; in man, (a) skin and mucosa: seborrhoeic and desquamative dermatitis of the mouth and eyes which may spread to the face, scalp, neck and loins; intertrigo of the breasts and inguinal region in women; stomatitis and glossitis; (b) nervous system: irritability, depression, somnolence, nausea, impairment of sensitivity to vibration and positional change; very rarely peripheral neuritis[37].

Spontaneous vitamin B_6 deficiency is rare in man. Among 300 infants who received only about 60 µg vitamin B_6 per litre of formula milk (as a result of a new sterilization process) hyperacusis, nervousness and epileptiform convulsions were observed[39]. Of possible genetic origin are the pyridoxine-dependent convulsions seen in infants (generalized convulsions with no clinical or electroencephalographic peculiarities appearing in the first days of life and occasionally developing into status epilepticus during the following weeks)[39–40]; these are probably due to an unsatisfied vitamin B_6 requirement or to a disturbance of vitamin B_6 utilization. Similar causes are probably at the root of the pyridoxine-deficiency anaemia and the pyridoxine-sensitive anaemia seen in man[39, 41, 42], but in contrast to the central nervous disturbances these are observed almost solely among adults. In pyridoxine-deficiency anaemia (a hypochromic microcytic anaemia with increased serum iron and organ haemosiderosis) there is a disturbance of δ-aminolaevulinic acid synthesis, with consequent reduction in the amount of protoporphyrin formed; this disease is also probably of genetic origin. In pyridoxine-sensitive anaemia (symptoms as in the deficiency anaemia but often including enlargement of the liver and spleen) there is a complex disturbance of porphyrin metabolism resembling that in sideroachrestic anaemia.

It is uncertain whether vitamin B_6 deficiency in man causes dental caries[43] or the formation of oxalate stones in the urinary tract[44].

Cystathionuria is probably due to a defect in the linkage of the coenzyme pyridoxal phosphate to the apoenzyme of homoserine dehydratase (cystathionase)[45].

Biochemically, vitamin B_6 deficiency is recognizable (a) by the increased excretion of xanthurenic acid and other tryptophan metabolites in urine, especially following oral doses of tryptophan (tryptophan loading test)[20]; this disturbance of tryptophan metabolism appears after only one week on a vitamin B_6-deficient diet[46]; (b) by a lowered pyridoxal phosphate level in the blood and a greatly reduced vitamin B_6 and pyridoxic acid excretion in the urine[47]; (c) by a reduced transaminase content of the serum[47] and erythrocytes[48]; (d) by an increased urinary oxalic acid excretion[44, 49] and a lowered urinary taurine excretion[49].

Treatment

When the vitamin B_6 deficiency is purely alimentary, daily doses of the vitamin at the level of the normal requirement suffice. In pyridoxine-dependent convulsions in infants pyridoxine should be given parenterally at the rate of 2–15 mg per day[40]; in pyridoxine-deficiency anaemia the dose should be at least 10 mg per day[41] and in pyridoxine-sensitive anaemia at least 500 mg per day[41]. Daily pyridoxine supplements of 10–15 mg may be helpful in overcoming disturbances of pregnancy such as severe vomiting and toxaemia, particularly when the diet is poor[50]. Pyridoxine doses of 100 mg have been recommended for the treatment of radiation sickness[51]. The success of treatment with pyridoxine is conditional on there being no disturbance of the conversion into pyridoxal (by the action of pyridoxine dehydrogenase) in the body[9].

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Nicotinic acid[1–3] (for references see page 478)

Chemistry

For structure and properties of nicotinic acid and related compounds see the table opposite.

Assay

Biological[4]. Black tongue curative test in dogs, growth test in chicks.

Microbiological[5]. With *Lactobacillus plantarum* (earlier *L. arabinosus*) or *Tetrahymena pyriformis* for nicotinic acid and nicotinamide.

Chemical[6]. In pure solution spectrophotometrically, polarographically, by mass analysis, colorimetrically by the bromcyanogen (König) reaction or fluorometrically (the latter method particularly suitable for organs). Nicotinic acid and its metabolites can be separated by chromatographic methods. NAD and NADP must be hydrolysed before being determined as nicotinic acid; they can be estimated directly by the spectrophotometric or fluorimetric method through their blue fluorescence in alkaline solution.

Unit

No international unit; by weight.

Biogenesis[7, 8]

In plants nicotinic acid arises by condensation of 3- and 4-carbon units. In animals, fungi and a few bacteria (for instance, *Xanthomonas pruni*) nicotinic acid is formed from tryptophan by the action of thiamine, riboflavin and vitamin B_6 (see diagram on page 475). It is less likely that nicotinamide is formed directly from nicotinic acid than by the hydrolysis of nicotinamide dinucleotides. The latter compounds are formed in erythrocytes, liver, yeast, etc. in accordance with the following scheme:

5-Phosphoribosyl 1-pyrophosphate
Nicotinic acid ⟶ Nic

Nicotini
(Deamid

Nicotinamide adenine dinucleotide phosphate ←ATP— Nicotin
(NAD)

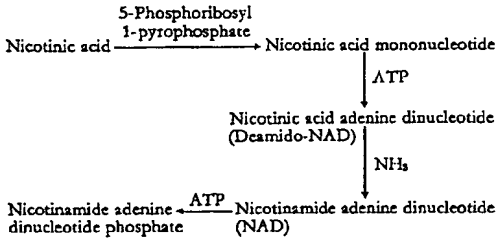
Intake and excretion

In the USA the daily average diet co
tryptophan and 8–17 mg nicotinic acid[9]
phan are readily absorbed in the intesti
probably synthesized from tryptophan
though it is unlikely that the human boc
In the body about 1 mg nicotinic acid is
tryptophan[1, 2]; the enzymes required fo
in the liver and erythrocytes. This con
nicotinic acid is increased in pregnancy[1]
The nicotinic acid content of whole b
litre and consists almost wholly of dinuc
The serum contains about 0.02–0.05 mg
also page 610). The nicotinic acid level in
when nicotinic acid is given parenteral

Structure and properties of nicotinic acid and

Compound	Formula mol. w
Nicotinic acid* (niacin, pyridine-3-carboxylic acid, vitamin PP)	$C_6H_5NO_2$ 123.11
Nicotinamide* (nicotinic acid amide, niacinamide, pyridine-3-carboxylamide, vitamin PP)	$C_6H_6N_2O$ 122.13
1-Methylnicotinamide (N_1-methylnicotinamide)	$C_7H_9N_2O^+$ 137.16
1-Methyl-6-pyridone-3-carboxylamide (N_1-methyl-2-pyridone-5-carboxylamide)	$C_7H_8N_2O_2$ 152.15
Nicotinamide adenine dinucleotide (NAD; reduced form $NADH_2$)	See page
Nicotinamide adenine dinucleotide phosphate (NADP; reduced form $NADPH_2$)	See page

* Trivial names recommended by the Commi
national Union of Biochemistry [*Biochim. bio*



Intake and excretion

In the USA the daily average diet contains about 500–1000 mg tryptophan and 8–17 mg nicotinic acid[9]. Nicotinic acid and tryptophan are readily absorbed in the intestinal tract. Nicotinic acid is probably synthesized from tryptophan by the intestinal bacteria, though it is unlikely that the human body makes use of this source. In the body about 1 mg nicotinic acid is formed from every 60 mg tryptophan[1,2]; the enzymes required for this transformation occur in the liver and erythrocytes. This conversion of tryptophan into nicotinic acid is increased in pregnancy[10].

The nicotinic acid content of whole blood is about 4–10 mg per litre and consists almost wholly of dinucleotides in the blood cells. The serum contains about 0.02–0.05 mg of free nicotinic acid[5] (see also page 610). The nicotinic acid level in the blood increases rapidly when nicotinic acid is given parenterally; oral doses of nicotinic acid or tryptophan raise the dinucleotide content of the erythrocytes. In the form of dinucleotides, nicotinic acid occurs in all tissues, particularly the liver. The human liver contains on the average 65 mg nicotinic acid[11].

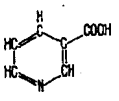
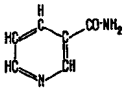
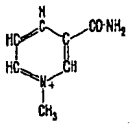
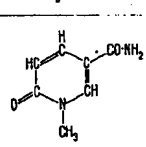
In the liver, nicotinamide but not nicotinic acid is methylated to 1-methylnicotinamide with *S*-adenosylmethionine as methyl-group donor and is further oxidized to 1-methyl-2-pyridonecarboxylamide[6]. These compounds are excreted in the urine (per day normally 5–8 mg 1-methylnicotinamide and 7–10 mg 1-methyl-2-pyridonecarboxylamide as well as about 1 mg nicotinic acid[2]; see also page 676). After oral doses of 10–150 mg nicotinamide per day, on the average 57% has been found in the urine, consisting of 10–30% 1-methylnicotinamide and 70–90% 1-methyl-2-pyridonecarboxylamide[12]. Following high doses of nicotinic acid but not nicotinamide, nicotinic acid is also excreted in the urine bound to glycine (as nicotinuric acid).

Function

The active forms of nicotinic acid are the nicotinamide dinucleotides NAD and NADP. These are coenzymes (cosubstrates) of numerous dehydrogenases, particularly in fermentation, glycolysis and other reactions. They are responsible also for hydrogen transport within the cell, $NADPH_2$ providing the hydrogen necessary for biosynthesis while $NADH_2$ transports this hydrogen to the enzymes of the respiratory chain. The synthesis of ATP utilizes the reaction with oxygen to form water (oxidative phosphorylation); for details see pages 403–404.

Nicotinic acid but not nicotinamide has an inhibiting effect at high dosage on the synthesis of lipids, particularly cholesterol, but

Structure and properties of nicotinic acid and related compounds

Compound	Formula and mol. wt.	Structure	Physical properties	Occurrence and activity
Nicotinic acid* (niacin, pyridine-3-carboxylic acid, vitamin PP)	$C_6H_5NO_2$ 123.11		White crystals, acid taste, moderately soluble in water and ethanol, stable to heat and oxidation M. p. 234–237 °C	In plant and animal tissues; component of NAD and NADP
Nicotinamide* (nicotinic acid amide, niacinamide, pyridine-3-carboxylamide, vitamin PP)	$C_6H_6N_2O$ 122.13		White crystals, salty taste, soluble in water and ethanol, stable to heat and oxidation M. p. 128–131 °C	In plant and animal tissues; component of NAD and NADP
1-Methylnicotinamide (N_1-methylnicotinamide)	$C_7H_9N_2O$ 137.16			In urine; metabolite of nicotinic acid
1-Methyl-6-pyridone-3-carboxylamide (N_1-methyl-2-pyridone-5-carboxylamide)	$C_7H_8N_2O_2$ 152.15		White crystals, soluble in water and ethanol M. p. 212–214 °C	In urine; metabolite of nicotinic acid
Nicotinamide adenine dinucleotide (NAD; reduced form $NADH_2$)	See page 344	See page 344	Colourless powder, soluble in water, insoluble in ethanol	In all animal and plant cells; coenzyme of many dehydrogenases
Nicotinamide adenine dinucleotide phosphate (NADP; reduced form $NADPH_2$)	See page 344	See page 344	Colourless powder, soluble in water, insoluble in ethanol	In all animal and plant cells; coenzyme of many dehydrogenases

* Trivial names recommended by the Commission on Biochemical Nomenclature of the International Union of Pure and Applied Chemistry and the International Union of Biochemistry [*Biochim. biophys. Acta (Amst.)*, 107, 1 (1965)].

the mechanism of this effect is obscure[13]; the primary action of nicotinic acid is possibly the liberation of free fatty acids blocked in the tissues[14].

Requirements and deficiency symptoms

The nicotinic acid requirement can also be met by tryptophan, 60 mg of which corresponds to 1 mg nicotinic acid. The requirement of nicotinic acid depends on the calorie intake. The minimum requirement for preventing pellagra is 4.4 mg per 1000 kcal, or 9 mg for adults whose daily calorie intake is below 2000 kcal[9]. For children and adults the Food and Nutrition Board (USA)[9] recommend a daily intake of 6.6 mg per 1000 kcal; during pregnancy this should be increased by 2 mg per day, during lactation by 7 mg per day (see page 494). The same daily intake of 6.6 mg/1000 kcal for children and adults is recommended by the Joint FAO/WHO Expert Group[22] (see page 493); for infants the latter accept that breast feeding by well-nourished mothers will supply adequate niacin equivalents.

Good sources of nicotinic acid are yeast, liver, lean meat, groundnuts and leguminous plants (see pages 499–515). Plant proteins contain 0.8–1.4% tryptophan, animal proteins about 1.3% (see page 516). Maize is low in both nicotinic acid and tryptophan; nicotinic acid is also present in the combined form so that it is not available to the organism[15]. During the roasting of coffee considerable amounts of nicotinic acid are formed from trigonelline[16].

Nicotinic acid deficiency causes pellagra, the development of which is favoured by sunlight and heavy physical work. Alimentary nicotinic acid deficiency is common in areas where maize constitutes the principal foodstuff. Pellagra occasionally occurs in chronic alcoholism, cirrhosis of the liver, chronic diarrhoea, diabetes and neoplasias. In the presence of carcinoid tumours up to 60% (normally 1%) of the body's tryptophan is converted into serotonin, so that it is no longer available as a source of nicotinic acid[17]. Treatment with isoniazid can cause inhibition of the activity of pyridoxal phosphate and thus interfere with the synthesis of nicotinic acid from tryptophan. This synthesis is possibly also impaired by diets containing large amounts of leucine[18].

The following are the symptoms of pellagra: (a) A dark red erythema appearing symmetrically on the extremities, face, neck and all other regions exposed to air and light; the skin finally becomes dry, fissured, atrophic and brown-coloured. The lesions are marked by atrophy of the superficial layers of the corium with dilatation of the blood vessels, keratinization of the epidermis and a tendency for the latter to separate from the corium. Wounds of any kind exacerbate these symptoms. (b) Chronic inflammation of the mucosa and intestinal tract (stomatitis, glossitis, gastritis with low acid secretion); profuse and often bloody diarrhoea. (c) Emotional disturbances (delirium, hallucinations, confused mental states). Neurological disturbances, if present, are probably due to simultaneous deficiency of other vitamins since these symptoms have not been observed in experimental nicotinic acid deficiency[2].

The biochemical signs of nicotinic acid deficiency are the following: in pellagra a urinary excretion of 1-methylnicotinamide plus 1-methyl-2-pyridonecarboxylamide of usually less than 2 mg per day. Within 30–60 days the excretion of these metabolites falls to a minimum value and then remains constant; shortly after this minimum value is reached, the first clinical signs of deficiency appear[2]. On a standard diet (10 mg nicotinic acid plus 1000 mg tryptophan) the excretion of nicotinic acid metabolites is less than 3.0 mg in pellagra patients and 7–37 mg in healthy persons[19]. In nicotinic acid deficiency the concentration of nicotinamide dinucleotides in the muscles and liver falls, but not that in the erythrocytes[2, 20].

Treatment

In severe nicotinic acid deficiency, 300–500 mg nicotinamide should be given in daily oral doses of 50–100 mg; if there is difficulty in swallowing, 100 mg nicotinamide should be given three times per day intramuscularly[2]. Nicotinic acid should not be given intravenously in doses exceeding 25 mg owing to the danger of anaphylactic shock. In high doses nicotinic acid but not nicotinamide causes marked dilatation of the vessels and particularly of the capillaries and vessels of the upper half of the body; this is of therapeutic use in disturbances of the peripheral circulation. Nicotinic acid can be used to lower the serum cholesterol level and for this purpose is usually given at the rate of 1 g three times per day[21].

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Folic acid group[1-3]

Chemistry[4]

For structure and properties of folic acid and related compounds see the table on pages 480–481.

Assay

Biological. Curative test on chickens[5].

Microbiological[1, 6]. With *Lactobacillus casei* (total folic acid activity: pteroylglutamic acid, pteroyltriglutamic acid and higher conjugates, reduced folic acid including 5-methyltetrahydropteroylglutamic acid); with *Streptococcus faecalis* (pteroylglutamic acid, reduced folic acid, but not 5-methyltetrahydropteroylglutamic acid); with *Pediococcus cerevisiae* (reduced folic acid). In biological material, particularly foodstuffs, the conjugates can also be broken down enzymatically.

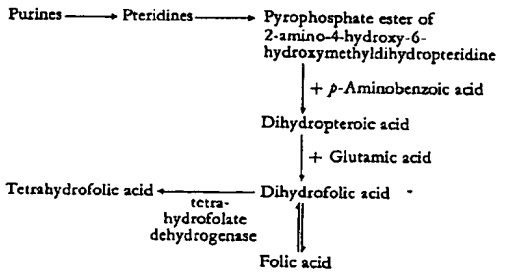
Chemical[7]. Photometrically after fission with zinc or potassium permanganate and subsequent diazotization; in pure solution also spectrophotometrically or polarographically.

Units

No international unit; by weight.

Biogenesis[8]

Folic acid is synthesized by higher plants, by micro-organisms (intestinal flora) and in animal tissues[9], probably in accordance with the following scheme:



The biosynthesis of dihydrofolic acid is inhibited by sulphonamides[10]. Folic acid antagonists like aminopterin and substances with a structure resembling pyramidine (e.g., primidone and pyrimethamine) inhibit tetrahydrofolate dehydrogenase and thereby the formation of tetrahydrofolic acid.

Intake and excretion

In USA the daily diet contains about 150–200 µg folic acid activity[11, 12]; this includes only about 20 µg pteroylglutamic acid[12].

The folic acid conjugates are hydrolys
tract. Both the folic acid derived from
the intestinal flora are actively absorbed
testine; large amounts also diffuse passi
wall[13].

On the folic acid content of blood,
see page 610. The maximum folic acid
is attained 2–4 hours after an oral dose
life of intravenous doses in the serum is
administration of folic acid has no effec
level; the exogenous folic acid is reduc
in small amounts. The total folic acid
has been estimated at 12–15 mg[16]; of
contained in the liver[17]. In serum[14, 19],
folic acid is predominantly present in
hydropteroylglutamic acid. The body's
cient to prevent the appearance of clinic
4–5 months[19]. After an intravenous dos
active folic acid and 10-formyltetrahydr
been found in the urine as well as unc
pteroylglutamic acid originating from

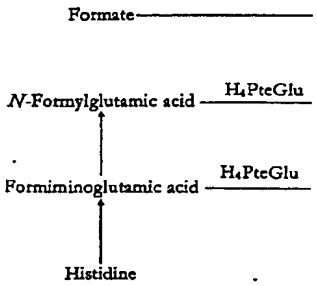
Function[23]

Tetrahydrofolic acid ($H_4PteGlu$) is a
carbon units. These units arise mainly fr
are required for the synthesis of purin
scheme below and pages 433 and 436).

On account of the importance of folic
acid synthesis it is understandable that
essential part in all processes of cell divi
poiesis (stimulation of the reticulocytes)
primary defect is probably a disturbanc
in the nucleus during cell division. The
taining normal pregnancy is still obscu
alters the action of the ovarial hormone

Requirements

The folic acid requirement is not kno
minimum requirement of adults is proba
sufficient to maintain the serum level[21]
requirement of about 5–20 µg[28] or mor
creased in pregnancy (possibly 200–40
mester when there is a deficiency of fola
tion Board (USA)[24] recommend a daily
0.8 mg for pregnant women, and 0.5 mg
are amounts determined in foods by
page 494).



Treatment

Treatment of folic acid deficiency
should be adjusted as closely as possibl
ciency[34]. In megaloblastic anaemia due
vitamin, 0.25 mg folic acid per day is
blood picture[35]. Doses of folic acid ex
required to prevent the appearance o
vitamin B_{12} deficiency but are inadeq
system from subacute degenerative
employing folic acid antagonists (ca
given in reduced form (for example
pregnancy, prophylactic doses of 0.1–
mended[16].

The folic acid conjugates are hydrolysed in the upper intestinal tract. Both the folic acid derived from the diet and that formed by the intestinal flora are actively absorbed in all parts of the small intestine; large amounts also diffuse passively through the intestinal wall[13].

On the folic acid content of blood, erythrocytes and leucocytes see page 610. The maximum folic acid concentration in the serum is attained 2–4 hours after an oral dose of the vitamin[14]. The half-life of intravenous doses in the serum is about 6–10 minutes[15]. Oral administration of folic acid has no effect on the serum folinic acid level; the exogenous folic acid is reduced in the tissues and stored in small amounts. The total folic acid content of the human body has been estimated at 12–15 mg[16]; of this amount about 7 mg is contained in the liver[17]. In serum[18,19], erythrocytes[20] and liver[21], folic acid is predominantly present in the form of 5-methyltetrahydropteroylglutamic acid. The body's stores are probably sufficient to prevent the appearance of clinical deficiency symptoms for 4–5 months[19]. After an intravenous dose of tagged folic acid, radioactive folic acid and 10-formyltetrahydropteroylglutamic acid have been found in the urine as well as untagged 5-methyltetrahydropteroylglutamic acid originating from the tissues[22].

Function[23]

Tetrahydrofolic acid (H_4PteGlu) is an important carrier of one-carbon units. These units arise mainly from histidine and serine and are required for the synthesis of purines and methionine (see the scheme below and pages 433 and 436).

On account of the importance of folic acid for purine and nucleic acid synthesis it is understandable that the vitamin should play an essential part in all processes of cell division, particularly in haemopoiesis (stimulation of the reticulocytes). In folic acid deficiency the primary defect is probably a disturbance of the doubling of DNA in the nucleus during cell division. The role of the vitamin in maintaining normal pregnancy is still obscure, but it is possible that it alters the action of the ovarial hormones on the uterus.

Requirements

The folic acid requirement is not known for certain[24]. The daily minimum requirement of adults is probably about 50 µg, an amount sufficient to maintain the serum level[25]. Infants have a minimum requirement of about 5–20 µg[26] or more[27]. The requirement is increased in pregnancy (possibly 200–400 µg per day in the 3rd trimester when there is a deficiency of folate[28]). The Food and Nutrition Board (USA)[24] recommend a daily intake of 0.4 mg for adults, 0.8 mg for pregnant women, and 0.5 mg for lactating women; these are amounts determined in foods by *Lactobacillus casei* assay (see page 494).

Good sources of folic acid are liver, kidneys, dark-green leaf vegetables, yeast (see pages 499–515). The folic acid content of fresh breast and cow's milk is sufficient to meet the requirement in infancy[29]. Folic acid is very sensitive to heat and 50–95% of the activity is destroyed by cooking.

Deficiency[30,31]

The following are causes of folic acid deficiency:

(a) Inadequate dietary intake (infants, alcoholics, cirrhotic patients)
(b) Disturbances of intestinal absorption (malabsorption syndrome, resection of the jejunum, haemochromatosis)
(c) An increased requirement (pregnancy, chronic haemolytic anaemia, malignant disease)
(d) Disturbances of folic acid metabolism (folic acid antagonists: see under 'Biogenesis' above).

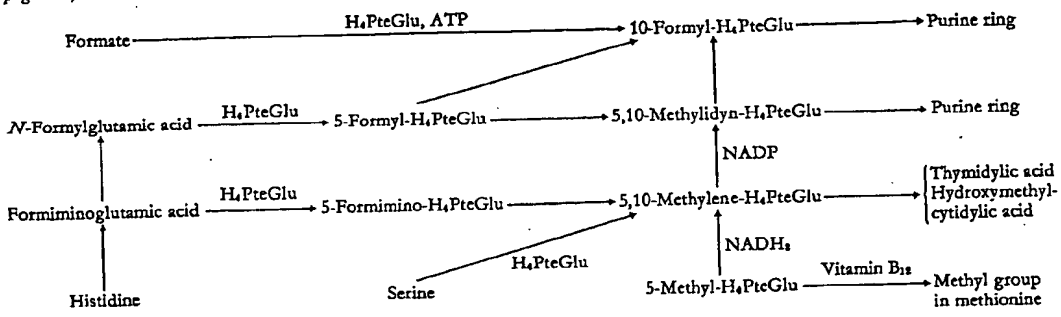
A daily intake of 5 µg folic acid or less results in deficiency of the vitamin[32]. The clinical symptoms are megaloblastosis of the bone marrow, macrocytic anaemia, leucopenia, excessive segmentation of the leucocytes, thrombocytopenia, glossitis and gastrointestinal disturbances. Biochemical symptoms of use in diagnosis are lowered folic acid contents of the serum and erythrocytes (see page 610) and the increased urinary excretion of formiminoglutamic acid after histidine loading (see page 670). Folic acid deficiency can also be diagnosed by a therapeutic test (0.05–0.2 mg folic acid per day for 10 days orally or, in malabsorption, intramuscularly)[2]. Other methods are measurement of the folic acid absorption using tritium-tagged folic acid and of the plasma folic acid clearance.

Deficiency symptoms appear in the following order[33]:

Lowered serum folic acid level (< 7–16 µg/l)	2 weeks
Excessive segmentation of the leucocytes	6–10 weeks
Increased excretion of formiminoglutamic acid	12–18 weeks
Lowered erythrocyte folic acid content	17 weeks
Macroelliptocytosis	18 weeks
Megaloblastosis of the bone marrow	19 weeks
Macrocytic anaemia	20 weeks

Folic acid deficiency is a common cause of megaloblastic anaemia in pregnancy. It is also often seen in children in the tropics, the main symptoms being loss of appetite and slow growth rather than megaloblastic anaemia[31].

Secondary folic acid deficiency in vitamin B_{12} deficiency is probably due to blocking of tetrahydrofolic acid regeneration from 5-methyltetrahydrofolic acid, a step requiring vitamin B_{12}[19].



Treatment

Treatment of folic acid deficiency with doses of the vitamin should be adjusted as closely as possible to the severity of the deficiency[34]. In megaloblastic anaemia due to dietary deficiency of the vitamin, 0.25 mg folic acid per day is sufficient to normalize the blood picture[35]. Doses of folic acid exceeding 0.1 mg per day are required to prevent the appearance of anaemia in patients with vitamin B_{12} deficiency but are inadequate to protect the nervous system from subacute degenerative changes[24,36]. In treatment employing folic acid antagonists (cancer) folic acid should be given in reduced form (for example as folinic acid)[37]. During pregnancy, prophylactic doses of 0.1–0.5 mg folic acid are recommended[18].

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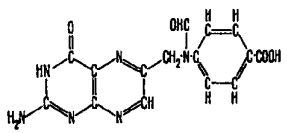
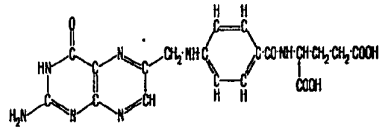
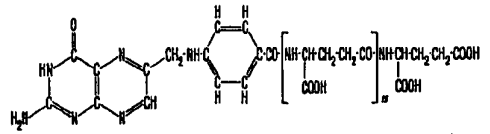
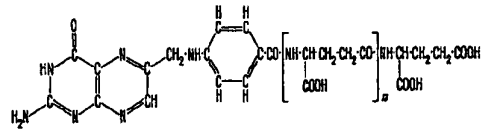
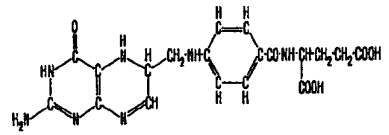
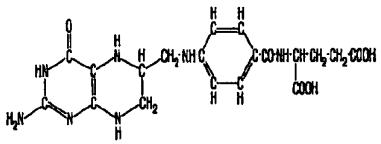
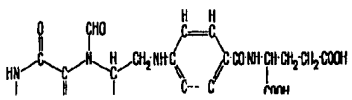
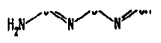
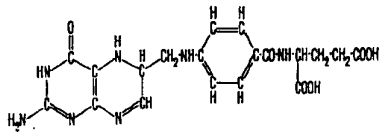
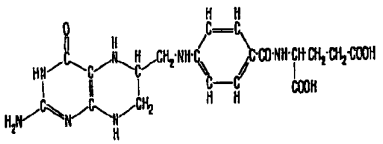
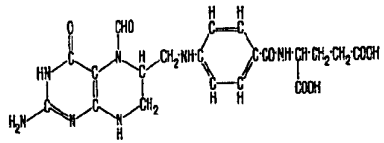
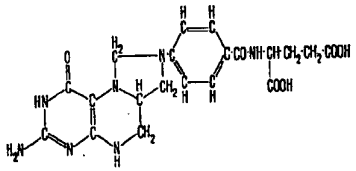
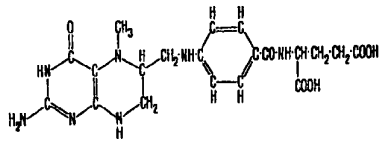
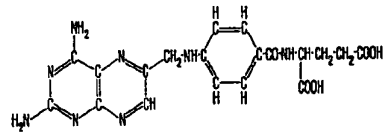
5 O'Dell and Hogan, *J. biol. Chem.*, 149, 323 (1943).
6 Baker and Sobotka, *Advanc. clin. Chem.*, 5, 173 (1962).
7 Gstirner, F., *Chemisch-physikalische Vitaminbestimmungsmethoden*, 5th ed., Enke, Stuttgart, 1965, page 188.

(continued on page 482)

Structure and properties of folic acid and related compounds

Names*	Formula and mol. wt.	Structure	Physical properties	Occurrence and biological properties
Rhizopterin (SLR factor)	$C_{15}H_{18}N_8O_4$ 340.30		Light-yellow platelets	In fermentation juice of *Rhizopus nigrans*. Weak folic acid activity
Pteroylglutamic acid (folic acid, folacin, vitamin B_c, *p*-[2-amino-4-oxo-dihydropteridyl-(6)]-methylaminobenzoyl-L-glutamic acid)	$C_{19}H_{19}N_7O_6$ 441.41		Orange-yellow needles or platelets, odourless and tasteless $[\alpha]+20°$ in 0.1-N NaOH	In liver, yeast, green leaves. Growth factor for *Lactobacillus casei*, *Streptococcus faecalis R* and other micro-organisms. Anti-anaemic properties
Pteroyltriglutamic acid, $PteGlu_3$ (teropterin)	$C_{29}H_{32}N_8O_{12}$ 699.64	 $n=2$	Light-yellow amorphous powder	In micro-organisms; formed in fermentations induced by corynebacteria. Weak folic acid activity
Pteroylheptaglutamic acid, $PteGlu_7$ (vitamin B_c conjugate)	$C_{49}H_{61}N_{13}O_{24}$ 1216.11	 $n=6$	Orange crystals	In yeast. Microbiologically inactive; presumably the form in which pteroylglutamic acid is stored
Dihydropteroylglutamic acid, $H_2PteGlu$ (dihydrofolic acid, DHF)	$C_{19}H_{21}N_7O_6$ 443.42		Light-yellow amorphous powder	Intermediary metabolite
Tetrahydropteroylglutamic acid, $H_4PteGlu$ (tetrahydrofolic acid, THF)	$C_{19}H_{23}N_7O_6$ 445.44		Pale cream-coloured powder, oxidizing in air, unstable to light, particularly in solution (-)-L-Form: $[\alpha]_D^{17}-16.9°$	Active form of folic acid
5-Formyltetrahydropteroylglutamic acid (citrovorum factor, folinic acid, leucovorin)	$C_{20}H_{23}N_7O_7$ 473.45		Colourless crystals $[\alpha]_D-15.1°$ (natural factor) $[\alpha]_D^{25}+16.76°$ (racemate,	In micro-organisms. Growth factor for *Leuconostoc citrovorum*, *Lactobacillus casei*, *Streptococcus faecalis*, *Lactobacillus arabinosus*; carrier of

		 $n = 6$		
Dihydropteroylglutamic acid, $H_2PteGlu$ (dihydrofolic acid, DHF)	$C_{19}H_{21}N_7O_6$ 443.42		Light-yellow amorphous powder	Intermediary metabolite
Tetrahydropteroylglutamic acid, $H_4PteGlu$ (tetrahydrofolic acid, THF)	$C_{19}H_{23}N_7O_6$ 445.44		Pale cream-coloured powder, oxidizing in air, unstable to light, particularly in solution (−)-L-Form: $[\alpha]_D^{27} -16.9°$	Active form of folic acid
5-Formyltetrahydropteroylglutamic acid (citrovorum factor, folinic acid, leucovorin)	$C_{20}H_{23}N_7O_7$ 473.45		Colourless crystals $[\alpha]_D$ −15.1° (natural factor) $[\alpha]_D^{25}$ +16.76° (racemate, synthetic factor)	In micro-organisms. Growth factor for *Leuconostoc citrovorum*, *Lactobacillus casei*, *Streptococcus faecalis*, *Lactobacillus arabinosus*; carrier of one-carbon units
5,10-Methylenetetrahydropteroylglutamic acid ('active formaldehyde')	$C_{20}H_{21}N_7O_6$ 457.45		Unstable in acid and neutral media	Carrier of one-carbon units
5-Methyltetrahydropteroylglutamic acid	$C_{20}H_{23}N_7O_6$ 459.47			In serum and liver. Carrier of one-carbon units
4-Aminopteroylglutamic acid (aminopterin)	$C_{19}H_{20}N_8O_5$ 440.42		Yellow needles	Antagonist of folic acid; inhibits cell division

* In accordance with the recommendations of the Commission on Biochemical Nomenclature of the IUPAC (IUPAC-IUB Commission on Biochemical Nomenclature [*Biochim. biophys. Acta (Amst.)*, 107, 11 (1965)] the names 'folic acid' and 'folates' should be used only as general designations for compounds of the group or mixtures of such compounds and not for any compound named on the basis of its structural formula.

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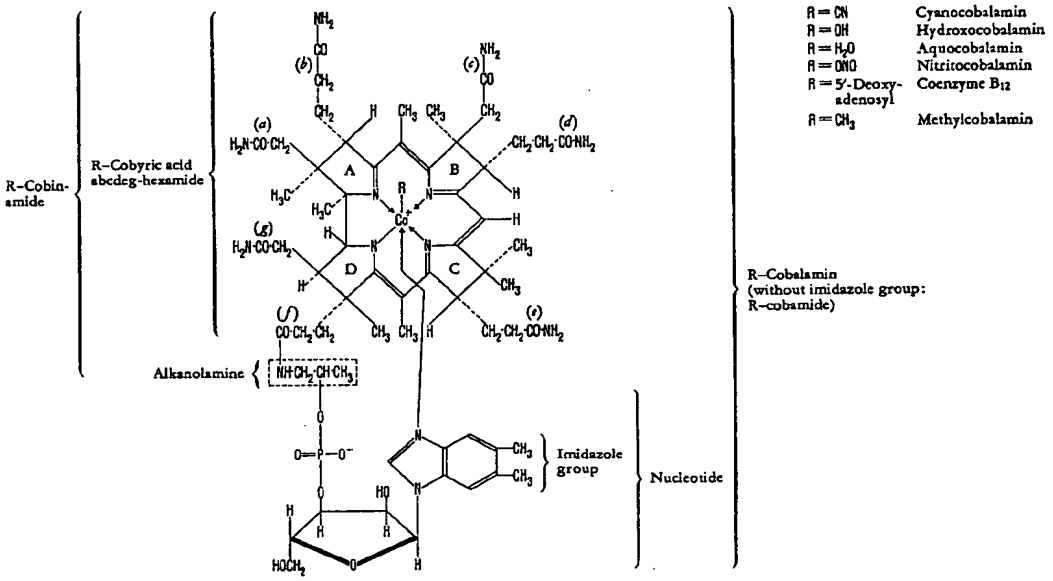
Vitamin B_{12} group (corrinoids)[1,2] (for references see page 485)

Chemistry[3]

All the complete B_{12} vitamins contain an α-glycosidic nucleotide. The imidazole nitrogen atom of this nucleotide can become co-ordinated under suitable conditions with the cobalt atom. In the case of the incomplete types either the alkanolamine and nucleotide portion, or simply the latter, is lacking; in some cases the nucleotide portion is β-glycosidic, when the imidazole nitrogen atom cannot become co-ordinated with the cobalt atom. The B_{12} coenzymes correspond to the complete B_{12} types but in place of the inorganic group they possess an organic group linked directly via a carbon atom to the cobalt atom. The complete and incomplete B_{12} types fairly stable to light and oxygen are presumably artefacts of the B_{12} coenzymes.



Names*	Formula and mol. wt.	Physical properties	Occurrence and biological properties
		Complete B_{12} types	
Vitamin B_{12} (cyanocobalamin, 5,6-dimethylbenzimidazolylcyanocobamide)	$C_{63}H_{88}N_{14}O_{14}PCo$ 1355.40	Red needles, stable on heating several hours at 100 °C. Spectral absorption in water: maxima at 278, 361, 550 nm	Occurs in nature as coenzyme. Can be isolated from animal tissues, many species of bacteria, sewage sludge, activated sludge. Stimulates maturation of erythrocytes in bone marrow; acts as *animal protein factor* in animals; promotes growth of many micro-organisms

* Trivial names recommended by the Commission on Biochemical Nomenclature of the International Union of Pure and Applied Chemistry and the International Union of Biochemistry [*Biochim. biophys. Acta (Amst.)*, 117, 285 (1966)].

Names*	
Aquocobalamin (vitamin B_{12b}, 5,6-dimethylbenzimidazolylaquocobamide) Hydroxocobalamin (vitamin B_{12a}, 5,6-dimethylbenzimidazolylhydroxocobamide)	C_{62} 1347
5-Methylbenzimidazolylcyanocobamide	
Benzimidazolylcyanocobamide	
5-Hydroxybenzimidazolylcyanocobamide (Factor III)	
Pseudovitamin B_{12} (adeninecyanocobamide)	
2-Methyladeninecyanocobamide (Factor A)	
Aetiocobalamin (Factor B, cyanocobinamide)	
5′-Deoxyadenosylcobalamin (coenzyme B_{12})	
Methylcobalamin	

* Trivial names recommended by the Com
national Union of Biochemistry [*Biochim. b*

Assay

Microbiological[1-4]. With bacteria: *Escl*
amide pseudo-vitamin B_{12}), *Lactobacil*
pseudo-vitamin B_{12}). With protozoa:
pseudo-vitamin B_{12}), *Ochromonas malha*

Chemical[5]. Spectrophotometrically o
solution; separation of the individua
current diffusion, column chromatogra

Isotopic dilution method. Tagging of v
or ^{60}Co.

Vitamin B_{12} Group

(For references see page 485)

Names*	Formula and mol. wt.	Physical properties	Occurrence and biological properties
Aquocobalamin (vitamin B_{12b}, 5,6-dimethylbenzimidazolylaquocobamide) Hydroxocobalamin (vitamin B_{12a}, 5,6-dimethylbenzimidazolylhydroxocobamide)	$C_{62}H_{90}N_{13}O_{15}PCo$ 1347.39	Red needles, aquo form in neutral solution, hydroxo form in alkaline solution Spectral absorption in water: maxima at 274, 350, 522 nm	Activity as for cyanocobalamin; depot form in the human body
5-Methylbenzimidazolylcyanocobamide		Red needles	In sewage sludge and activated sludge. Represents two-thirds of the cyanocobalamin activity in pernicious anaemia
Benzimidazolylcyanocobamide		Red needles	In sewage sludge and activated sludge. Represents two-thirds of the cyanocobalamin activity in pernicious anaemia
5-Hydroxybenzimidazolylcyanocobamide (Factor III)		Red needles	Inactivated sludge; weakly active in pernicious anaemia
Pseudovitamin B_{12} (adeninecyanocobamide)		Red needles	Inactivated sludge, faeces, stomach contents of ruminants; inactive in pernicious anaemia
2-Methyladeninecyanocobamide (Factor A)		Red needles	Inactivated sludge, faeces, stomach contents of ruminants; very weakly active in pernicious anaemia
Incomplete B_{12} types			
Aetiocobalamin (Factor B, cyanocobinamide)		Amorphous	Inactivated sludge, faeces, stomach contents of ruminants; antagonist of cayanocobalamin in the chick test
B_{12} coenzymes			
5′-Deoxyadenosylcobalamin (coenzyme B_{12})		Orange-yellow platelets, photosensitive	In many species of bacteria, in animal tissues (mainly liver). Biochemically active form of vitamin B_{12}. Growth promoting-activity for micro-organisms and chicks; activity in pernicious anaemia as for cyanocobalamin; depot action in the human body
Methylcobalamin		Orange-yellow platelets, photosensitive	In animal tissues (liver), blood serum. Coenzyme function

* Trivial names recommended by the Commission on Biochemical Nomenclature of the International Union of Pure and Applied Chemistry and the International Union of Biochemistry [*Biochim. biophys. Acta (Amst.)*, 117, 285 (1966)].

Assay

Microbiological[1, 4]. With bacteria: *Escherichia coli* (cobalamins, cobamide pseudo-vitamin B_{12}), *Lactobacillus leichmannii* (cobalamins, pseudo-vitamin B_{12}). With protozoa: *Euglena gracilis* (cobalamins, pseudo-vitamin B_{12}), *Ochromonas malhamensis* (cobalamins only).

Chemical[5]. Spectrophotometrically or polarographically in pure solution; separation of the individual compounds by countercurrent diffusion, column chromatography or ion exchange.

Isotopic dilution method. Tagging of vitamin B_{12} with ^{57}Co, ^{58}Co or ^{60}Co.

Unit

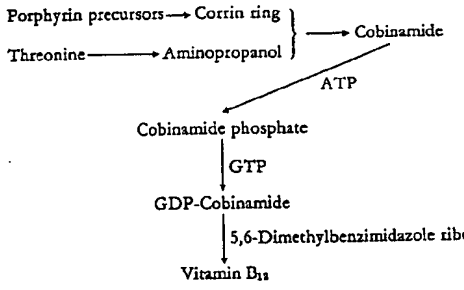
No international unit; by weight. 1 µg vitamin B_{12} = 11000 LLD (*Lactobacillus lactis* DORNER) Units = 1 USP Unit (liver extract). 1 USP Unit is the daily dose that produces a clinically and haematologically satisfactory response in true pernicious anaemia. From the standpoint of activity, 1000 LLD Units corresponds roughly to 1 ml of a good liver extract.

For International Reference Preparation see page 763.

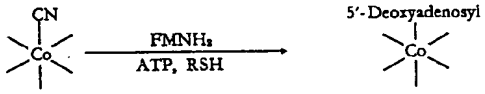
Biogenesis[6,7]

Vitamin B_{12} is synthesized by many species of bacteria, in particular the propionibacteria and *Aerobacter aerogenes*. It is possibly also formed in animal tissues[8].

The biosynthesis is in accordance roughly with the following scheme:



This process results in formation of the coenzyme forms of the vitamin; cyanocobalamin and hydroxocobalamin are probably only artefacts[9]; they can, however, be converted in animal tissues into the coenzymes:

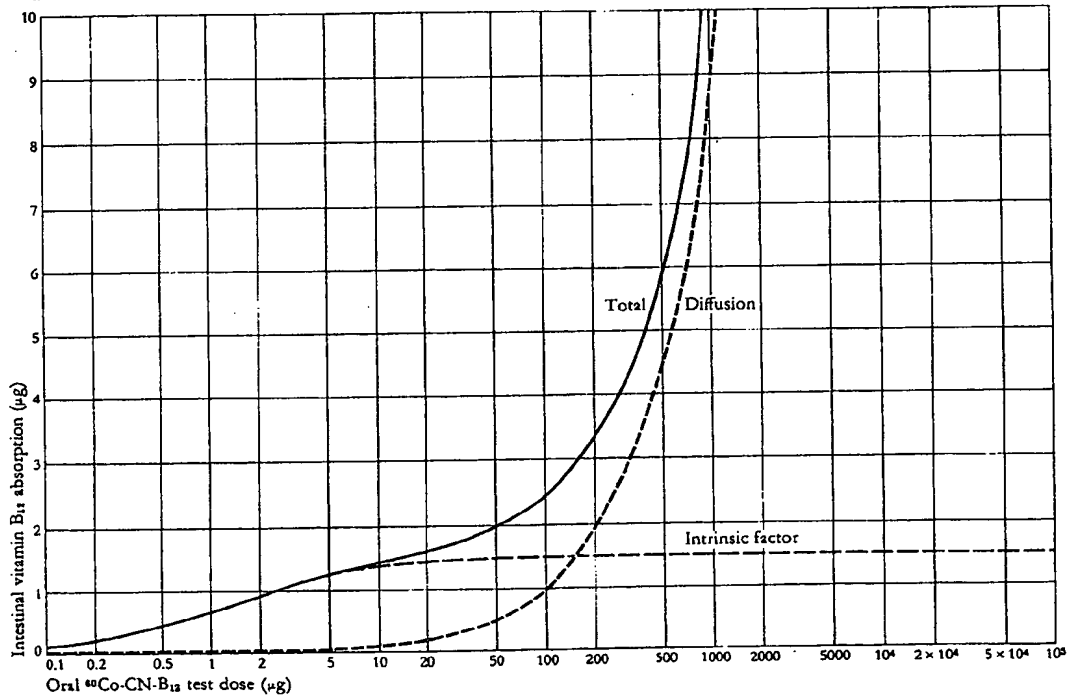


Intake and excretion

In the USA the average daily diet contains 15–30 μg vitamin B_{12}[10], of which perhaps 5 μg is absorbed. According to HEINRICH and WOLFSTELLER[12], of any dose of vitamin B_{12}, 1.5 μg is absorbed in the ileum with the aid of the so-called *intrinsic factor*, a mucoprotein of the gastric juice[11]; transport through the intestinal wall probably takes place together with the intrinsic factor[13]. In addition, there is passive diffusion through the intestinal wall[14] to an extent that increases logarithmically with increasing size of the dose up to a limiting value of 0.9% of the dose[12]. On a normal diet and three meals a day this means that 2–5 μg or more of vitamin B_{12} is absorbed daily[15] (see the figure below). Cyanocobalamin is rather more easily absorbed than coenzyme B_{12}[16]. Some 10–15 μg vitamin B_{12} is synthesized daily by bacteria in the human large intestine, and about the same quantity is excreted daily in the faeces; whether any of the quantity formed by bacterial synthesis is absorbed is doubtful[17].

The vitamin B_{12} content of the serum lies in the range 100–900 ng/l (see page 610); here the vitamin is mainly present as methylcobalamin, about 80% being bound to α-globulins (transcobalamin I). Exogenous vitamin B_{12} becomes bound for a short time to β-globulin (transcobalamin II)[18]. The half-life of intravenously administered cyanocobalamin in the serum is about 6 days[19]. Small doses of a few microgrammes of vitamin B_{12} are retained in the body but doses of a few milligrammes are rapidly excreted in the urine. Hydroxocobalamin given parenterally is retained in the body longer than cyanocobalamin or coenzyme B_{12}[16]. In the tissues the vitamin is probably stored as the coenzyme. The total body stores of the vitamin have been estimated at 2–5 mg (range 1–11 mg)[1,12,15]. The liver contains about 0.8 mg vitamin B_{12}[20] (the biological half-life of the vitamin in the liver is about 12 months[19]). 0.1% of the body's stores of vitamin B_{12} is excreted daily[15]. These stores probably suffice to prevent the appearance of clinical deficiency symptoms for 3–8 years[1,15]. Excretion of the vitamin is almost solely in the bile (see page 655), only very small amounts being found in the urine (0–0.27 μg per day)[1]. On SCHILLING's excretion test see pages 289 and 485.

Distribution of the total intestinal vitamin B_{12} absorption in healthy persons between absorption dependent on the intrinsic factor and absorption dependent on diffusion[12]



Function[7,21]

Vitamin B_{12} acts as coenzyme in va

Enzyme (reaction catalysed in brackets)	C
Methylaspartate mutase (glutamic acid ⇌ β-methylaspartic acid)	5'-Dec cobala
Methylmalonyl-CoA-mutase (methylmalonyl-CoA ⇌ succinyl-CoA)	5'-Dec cobala
Glycol dehydrogenase (ethyleneglycol ⇌ acetaldehyde, 1,2-propanediol ⇌ proplonaldehyde)	5'-Dec cobala
5-Methyltetrahydrofolate homocysteine transmethylase (Methionine formation)	Methy (prote as inte produ
Ribonucleotide reductase (DNA formation)	5'-Dec cobala
(Thiol oxidation)	Unkn

Inside the cell, vitamin B_{12} is locate is involved in a manner still largely un ably also lipid and carbohydrate meta the vitamin in the formation of methi in the regeneration of tetrahydrofolic B_{12} plays a very important part in haen reticulocytes) in that together with fol or indirectly in the formation of deox nucleotides. The significance of the vit is obscure (more is known of this role i is the part it plays in the maturation c growth[25].

Requirements

The daily requirement in adults app the absorption of 0.6–1.5 μg[15], but level is 5 μg[26] (see page 494). In pregr may be higher since the mother's rese the needs of the foetus; the Food an accordingly recommend a daily intake

The best sources of vitamin B_{12} (in tance) are liver, kidneys, meat and mil present in plants (see pages 499–515). than 30% of the vitamin[16].

Deficiency[27]

Vitamin B_{12} deficiency may arise fro

(a) Inadequate dietary intake (strict ve

(b) Absence or inadequacy of intrinsic anaemia, gastrectomy, gastroenter tric juice of antibodies to the intrin

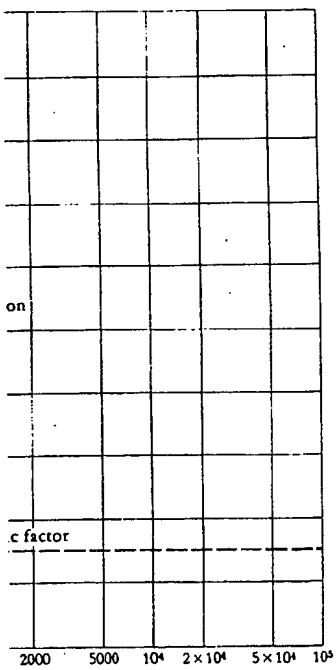
(c) Inadequate ileal absorption, (malal tuberculosis, ileal resection)

(d) Interference with absorption by ba in blind loops, diverticulosis of th fish tapeworm.

The following are the deficiency sym megaloblastosis of the bone marrow penia, glossitis, morphological change and (in contrast to folic acid deficiency) the axis cylinders of the spinal-cord ne of the pernicious anaemia is possibly in serum of many patients contains antib leading to atrophy of the gastric muc pernicious anaemia there is absence of a in children secretion of the intrinsic probably as a genetic defect – but acid

aily diet contains 15–30 μg vitamin B_{12}[10], absorbed. According to HEINRICH and ıse of vitamin B_{12}, 1.5 μg is absorbed in ıe so-called *intrinsic factor*, a mucoprotein port through the intestinal wall probably the intrinsic factor[13]. In addition, there ;h the intestinal wall[14] to an extent that with increasing size of the dose up to a the dose[12]. On a normal diet and three 2–5 μg or more of vitamin B_{12} is absorbed w). Cyanocobalamin is rather more easily $_{12}$[16]. Some 10–15 μg vitamin B_{12} is syn- in the human large intestine, and about ed daily in the faeces; whether any of the al synthesis is absorbed is doubtful[17]. t of the serum lies in the range 100–900 ıe vitamin is mainly present as methylco- bound to α-globulins (transcobalamin I). comes bound for a short time to β-globu- The half-life of intravenously adminis- he serum is about 6 days[19]. Small doses vitamin B_{12} are retained in the body but s are rapidly excreted in the urine. Hydro- erally is retained in the body longer than yme B_{12}[16]. In the tissues the vitamin is enzyme. The total body stores of the ited at 2–5 mg (range 1–11 mg)[1,12,16]. .8 mg vitamin B_{12}[20] (the biological half- iver is about 12 months[12]). 0.1% of the 12 is excreted daily[15]. These stores prob- : appearance of clinical deficiency symp- retion of the vitamin is almost solely in the ry small amounts being found in the urine SCHILLING's excretion test see pages 289

n the intrinsic factor and absorption dependent



Function[7,21]

Vitamin B_{12} acts as coenzyme in various biological reactions:

Enzyme (reaction catalysed in brackets)	Coenzyme	Occurrence
Methylaspartate mutase (glutamic acid ⇌ β-methylaspartic acid)	5′-Deoxyadenosyl-cobalamin	Bacteria
Methylmalonyl-CoA-mutase (methylmalonyl-CoA ⇌ succinyl-CoA)	5′-Deoxyadenosyl-cobalamin	Bacteria, animal tissues
Glycol dehydrogenase (ethyleneglycol ⇌ acetaldehyde, 1,2-propane-diol ⇌ propionaldehyde)	5′-Deoxyadenosyl-cobalamin	Bacteria
5-Methyltetrahydrofolate homocysteine trans-methylase (Methionine formation)	Methylcobalamin (protein complex) as intermediate product	Bacteria, animal tissues
Ribonucleotide reductase (DNA formation)	5′-Deoxyadenosyl-cobalamin	Bacteria, animal tissues
(Thiol oxidation)	Unknown	Bacteria, animal tissues

Inside the cell, vitamin B_{12} is located in the mitochondria[22] and is involved in a manner still largely unknown in protein and probably also lipid and carbohydrate metabolism. The role played by the vitamin in the formation of methionine is probably important in the regeneration of tetrahydrofolic acid (see page 437). Vitamin B_{12} plays a very important part in haemopoiesis (stimulation of the reticulocytes) in that together with folic acid it is involved directly or indirectly in the formation of deoxyribonucleotides from ribonucleotides. The significance of the vitamin in normal reproduction is obscure (more is known of this role in animals than in man[23]), as is the part it plays in the maturation of the spermatozoa[24] and in growth[25].

Requirements

The daily requirement in adults appears to be amply covered by the absorption of 0.6–1.5 μg[18], but a desirable daily absorption level is 5 μg[26] (see page 494). In pregnant women the requirement may be higher since the mother's reserves are largely exhausted by the needs of the foetus; the Food and Nutrition Board (USA)[26] accordingly recommend a daily intake of 8 μg (see page 494).

The best sources of vitamin B_{12} (in decreasing order of importance) are liver, kidneys, meat and milk; only small quantities are present in plants (see pages 499–515). Cooking destroys not more than 30% of the vitamin[15].

Deficiency[27]

Vitamin B_{12} deficiency may arise from the following causes:

(a) Inadequate dietary intake (strict vegetarians)

(b) Absence or inadequacy of intrinsic factor secretion (pernicious anaemia, gastrectomy, gastroenterostomy; presence in the gastric juice of antibodies to the intrinsic factor[28])

(c) Inadequate ileal absorption (malabsorption syndrome, ileitis, tuberculosis, ileal resection)

(d) Interference with absorption by bacteria (intestinal congestion in blind loops, diverticulosis of the small intestine) or by the fish tapeworm.

The following are the deficiency symptoms: macrocytic anaemia, megaloblastosis of the bone marrow, leucopenia, thrombocytopenia, glossitis, morphological changes in the gastrointestinal tract and (in contrast to folic acid deficiency) progressive degeneration of the axis cylinders of the spinal-cord neurones. In adults, the cause of the pernicious anaemia is possibly immunological processes (the serum of many patients contains antibodies to the intrinsic factor) leading to atrophy of the gastric mucosa. Whereas in adults with pernicious anaemia there is absence of acid secretion in the stomach, in children secretion of the intrinsic factor itself may be absent – probably as a genetic defect – but acid secretion normal[29].

Biochemical deficiency symptoms of use in diagnosis are the lowered vitamin B_{12} content of the serum (see page 610) and liver and the very much increased excretion of methylmalonic acid in the urine[30]. The amount of vitamin B_{12} absorbed from the diet can be estimated by measurement of the total faecal excretion over a period of 5 or more days, of the excretion in a single faecal sample using a double isotope technique with a nonabsorbable marker, of the hepatic uptake of radioactivity, of the urinary excretion after a flushing dose of untagged vitamin B_{12}, or of the whole-body retention of radioactivity. In malabsorption the test is repeated with intrinsic factor to see if this improves absorption; alternatively vitamin B_{12} and vitamin B_{12} intrinsic factor tagged with two different cobalt isotopes can be given simultaneously[31]. SCHILLING's test (0.5 μg tagged vitamin B_{12} orally followed one hour later by 1000 μg untagged vitamin B_{12} intramuscularly) gives the following values[32]: normal 8–34%, in pernicious anaemia 0–3.5% of the radioactivity in the 24-hour urine.

Treatment

The blood picture in pernicious anaemia shows an improvement with parenteral doses of as little as 0.1 μg vitamin B_{12} per day[33]; doses of 0.5–2.0 μg per day result in complete remission of the symptoms[34]. The body's reserves can be restored within 10 days by giving 5 doses each of 1000 μg subcutaneously. The usual maintenance dose is 100–400 μg parenterally once a month or 1000 μg orally twice a week[35]. Oral treatment with vitamin B_{12} plus intrinsic factor is not recommended since the body eventually develops resistance. Oral treatment with a vitamin B_{12}-peptide complex has been suggested[36].

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Biotin[1]

Chemistry

For structure and properties of biotin and related compounds see the table below.

Assay

Biological[3]. With *Saccharomyces cerevisiae*, *Lactobacillus casei*, *Lactobacillus arabinosus*, *Neurospora crassa*, etc.; in biological fluids preferably with *Ochromonas danica*.

Chemical. No methods in common use.

Unit

No international unit; by weight. 1 avidine unit = the smallest quantity that completely suppresses the growth-promoting effect in yeast of 1 μg biotin[4].

Biogenesis[2, 5]

Biotin is synthesized in plants (particularly in sprouting seeds) and various micro-organisms. Thus *Achromobacter* forms biotin from pimelyl-coenzyme A, cysteine and carbamyl phosphate; pimelyl-coenzyme A is formed from 3 molecules of malonyl-coenzyme A[6].

The carboxyl group of biotin is covalent and bound to the lysine residue of a protein. Biocytin is formed by the action of a proteinase on protein-bound biotin.

Intake and excretion

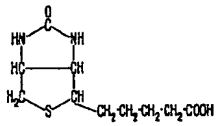
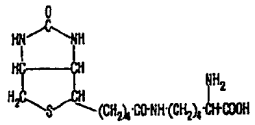
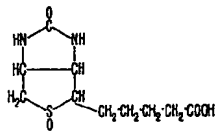
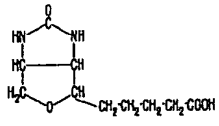
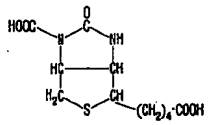
Biotin is formed by the intestinal flora in such large quantities that it is excreted in the faeces in an amount 2–5 times greater than the dietary intake[7]. The body appears to be capable of utilizing biotin formed in the intestine but to an unknown extent. The avidine present in raw egg-albumin combines with biotin, thus rendering it useless for the organism.

Biotin has been detected in whole blood and serum (see page 611) and in urine (see page 676). Small quantities are stored in the liver (about 0.2 mg) and in the brain[3, 8] (blood vessels 3–5 ng/g[9]). In the liver, biotin is mostly bound to protein (in rats 90%[6]). Little is known of the metabolism of biotin. In rats given injections of biotin 16% was found 4 hours later in the liver and 30% was excreted[6].

Function[5]

Biotin is essential for warm-blooded animals and some micro-organisms. It acts as coenzyme in CO_2-fixation and transcarboxylation reactions.

Structure and properties of biotin and related compounds

Names	Formula and mol. wt.	Structure	Physical properties	Occurrence	Activity
Biotin, *d*-biotin	$C_{10}H_{16}N_2O_3S$ 244.31		White needles, stable to heat, unstable to acids and alkalis M.p. 230–232 °C $[\alpha]_D^{16}$ +91° in 0.1-N NaOH	Various micro-organisms, for example yeasts, animal tissues, particularly liver, egg-yolk, plants	Growth factor for many bacteria, protozoa and probably all higher animals
Biocytin, *d*-biocytin (ε-*N*-biotinyl-L-lysine)	$C_{16}H_{28}N_4O_4S$ 372.49		M.p. 245–252 °C	Yeasts	Growth factor for various micro-organisms
Biotin sulphoxide, *d*-biotin 1-sulphoxide (AN factor)	$C_{10}H_{16}N_2O_4S$ 260.31			Cultures of *Aspergillus niger* and *Phycomyces blakesleeanus*	Growth factor for *Neurospora crassa*
Oxybiotin (oxobiotin)	$C_{10}H_{16}N_2O_4$ 228.25		M.p. 205–207 °C		5–30% of activity of biotin[2]
1′-*N*-Carboxybiotin (CO_2-biotin)	$C_{11}H_{16}N_2O_5S$ 288.33			Unstable intermediate	Active form of CO_2 in carboxylations

Biotin enzymes

Enzyme	Reaction catalys
Acetyl-CoA-carboxylase	acetyl-CoA + HCC ATP ⇌ malonyl-C ADP + P
β-Methylcrotonyl-CoA-carboxylase	β-methylcrotonyl- HCO_3^- + ATP ⇌ β-methylglutacony + ADP + P
Propionyl-CoA-carboxylase	propionyl-CoA + I + ATP ⇌ methylr nyl-CoA + ADP +
Methylmalonyl-CoA-carboxyl-transferase	methylmalonyl-Cc pyruvate ⇌ propic CoA + oxalacetate
Pyruvate carboxylase	pyruvate + HCO_3^- ⇌ oxalacetate + A

The carboxylation reactions catalysed the general form

$$\text{Biotin enzyme} + CO_2 + ATP \overset{Mg^{++}}{\rightleftharpoons} CC$$

$$CO_2\text{-biotin enzyme} + X \rightleftharpoons$$

(where X is for example β-methylcrot enzyme compound in which the CO_2 *N*-carboxybiotin in the table opposite acetyl-coenzyme A, on the other hand, of the biotin itself represents the active as 'diaminobiotin' is here assumed to a

The carboxylation of acetylcoenzym point in the biosynthesis of fatty acids an important role in many other reactic only in an indirect manner. Example deamination of aspartate, serine and th ation of serine in animals, reductive carboxylation of phosphoenol pyruva tryptophan metabolism, purine synth carbohydrate metabolism.

Certain biotin homologues (homo-o which the sulphur atom is replaced by vitamins[12].

Requirements and deficiency symp

The biotin requirement of man is un tin deficiency, doses of 150–300 μg pe deficiency symptoms[13, 14]; this amou diet[15].

Substances rich in biotin are liver, the vitamin is also present in vegetable 498–515).

Biotin deficiency is manifested[16] in pigs, chickens, man), hyperkeratosis ((man), loss of hair (rats, mice), loss Experimental biotin deficiency in ma appetite, nausea, muscular pain and lo taneous biotin deficiency has been rep raw eggs[17, 18] and also appears to be ass

Treatment

Biotin has been used to treat sebo children (LEINER's disease)[19], which i of biotin in the breast milk together to persistent diarrhoea.

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otin is covalent and bound to the lysine
tin is formed by the action of a proteinase

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eces in an amount 2–5 times greater than
body appears to be capable of utilizing
tine but to an unknown extent. The avi-
lbumin combines with biotin, thus ren-
rganism.
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rain [3, 6] (blood vessels 3–5 ng/g [3]). In the
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of biotin. In rats given injections of biotin
ater in the liver and 30% was excreted [5].

warm-blooded animals and some micro-
zyme in CO_2-fixation and transcarboxyl-

Occurrence	Activity
Various micro-organisms, for example yeasts, animal tissues, particularly liver, egg-yolk, plants	Growth factor for many bacteria, protozoa and probably all higher animals
Yeasts	Growth factor for various micro-organisms
Cultures of *Aspergillus niger* and *Phycomyces blakesleeanus*	Growth factor for *Neurospora crassa*
	5–30% of activity of biotin [2]
Unstable intermediate	Active form of CO_2 in carboxylations

Biotin enzymes

Enzyme	Reaction catalysed	Occurrence
Acetyl-CoA-carboxylase	acetyl-CoA + HCO_3^- + ATP ⇌ malonyl-CoA + ADP + P	Micro-organisms, chicken liver
β-Methylcrotonyl-CoA-carboxylase	β-methylcrotonyl-CoA + HCO_3^- + ATP ⇌ β-methylglutaconyl-CoA + ADP + P	Micro-organisms, rat liver mitochondria
Propionyl-CoA-carboxylase	propionyl-CoA + HCO_3^- + ATP ⇌ methylmalonyl-CoA + ADP + P	Swine heart, ox liver mitochondria
Methylmalonyl-CoA-carboxyltransferase	methylmalonyl-CoA + pyruvate ⇌ propionyl-CoA + oxalacetate	Propionibacteria, skeletal muscle of dogs
Pyruvate carboxylase	pyruvate + HCO_3^- + ATP ⇌ oxalacetate + ADP + P	Micro-organisms, liver mitochondria, rabbit kidneys

The carboxylation reactions catalysed by the biotin enzymes have the general form

$$\text{Biotin enzyme} + CO_2 + ATP \overset{Mg^{++}}{\rightleftharpoons} CO_2\text{-biotin enzyme} + ADP + P$$

$$CO_2\text{-biotin enzyme} + X \rightleftharpoons \text{Biotin enzyme} + CO_2\text{-}X$$

(where X is for example β-methylcrotonyl-CoA) via a CO_2-biotin enzyme compound in which the CO_2 is linked to biotin (see 1'-*N*-carboxybiotin in the table opposite)[10]. In the carboxylation of acetyl-coenzyme A, on the other hand, the carbamide carbon atom of the biotin itself represents the active CO_2[11]; a compound known as 'diaminobiotin' is here assumed to arise as intermediate product.

The carboxylation of acetylcoenzyme A is an important starting point in the biosynthesis of fatty acids (see page 424). Biotin plays an important role in many other reactions in which, however, it acts only in an indirect manner. Examples of such reactions are the deamination of aspartate, serine and threonine in bacteria, deamination of serine in animals, reductive carboxylation of pyruvate, carboxylation of phosphoenol pyruvate, carbamylation reactions, tryptophan metabolism, purine synthesis, protein synthesis and carbohydrate metabolism.

Certain biotin homologues (homo-oxybiotin; biotinsulphone, in which the sulphur atom is replaced by a sulphone group) act as antivitamins[12].

Requirements and deficiency symptoms

The biotin requirement of man is unknown. In experimental biotin deficiency, doses of 150–300 µg per day sufficed to suppress the deficiency symptoms[13,14]; this amount is provided by a normal diet[15].

Substances rich in biotin are liver, kidneys, yeast and egg-yolk; the vitamin is also present in vegetables, nuts and cereals (see pages 498–515).

Biotin deficiency is manifested[16] in nervous disturbances (rats, pigs, chickens, man), hyperkeratosis (rats), seborrhoeic dermatitis (man), loss of hair (rats, mice), loss of hair pigment (rats, mice). Experimental biotin deficiency in man results in lethargy, loss of appetite, nausea, muscular pain and localized paraesthesia[14]. Spontaneous biotin deficiency has been reported following a diet rich in raw eggs[17,18] and also appears to be associated with liver cirrhosis[18].

Treatment

Biotin has been used to treat seborrhoeic dermatitis in young children (LEINER's disease)[19], which is possibly due to a deficiency of biotin in the breast milk together with loss of the vitamin due to persistent diarrhoea.

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Pantothenic acid[1] (for references see page 489)

Chemistry

For structure and properties of pantothenic acid and related compounds see the table on page 488.

Assay

Biological. Growth test on chicks; microbiologically[2] with *Lactobacillus casei* or *L. plantarum* (*L. arabinosus*).

Chemical[4]. After hydrolysis by colorimetric determination of the β-alanine.

Unit

No international unit; by weight. 1 LIPMANN unit of coenzyme A = 2.4 µg of the pure compound (corresponding to 0.7 µg pantothenic acid)[5].

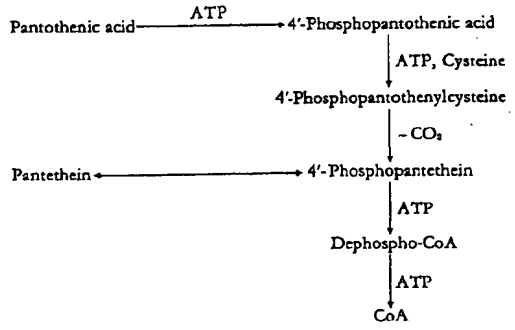
Biogenesis[6]

Pantothenic acid is formed by bacteria (e.g., *Escherichia coli*, *Bacterium linens*) from α-ketoisovaleric acid by addition of a one-carbon unit. The formation of pantothenic acid from pantoic acid and β-alanine is mediated by ATP (for instance in *Escherichia coli* and *Brucella abortus*) as follows:

$$\alpha\text{-Ketoisovaleric acid} \xrightarrow{HCHO} \alpha\text{-Ketopantoic acid} \xrightarrow{+\,2\,H} \text{Pantoic acid}$$

$$\text{Pantoic acid} + \beta\text{-Alanine} + ATP \rightarrow \text{Pantothenic acid} + \text{Pyrophosphate}$$

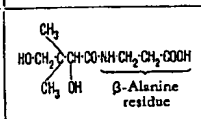
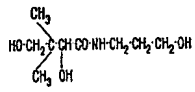
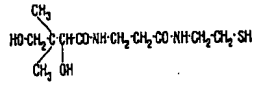
In animal tissues no pantothenic acid is formed; the vitamin is, however, incorporated in coenzyme A in both micro-organisms and animal tissues (for instance liver):



Intake and excretion

A 2500 kcal diet contains about 4–12 mg of free and 9–20 mg total pantothenic acid[7]. The vitamin is also synthesized in the human

Structure and properties of pantothenic acid and related compounds

Compound	Formula and mol. wt.	Structure	Physical properties	Occurrence and activity
Pantothenic acid (D-[+]-*N*-[α,γ-dihydroxy-ββ-dimethylbutyryl]-β-alanine, chick antidermatitis factor)	$C_9H_{17}NO_5$ 219.24	 β-Alanine residue	Yellow oil, unstable to heat, acids and alkalis $[\alpha]_D^{22}+37.5°$ Calcium salt: white crystals, stable to heat	Widely distributed in plants and animals. Growth factor for yeasts, many other micro-organisms and all higher animals; component of coenzyme A
Pantothenyl alcohol (panthenol, *N*-pantoyl-3-propanolamine)	$C_9H_{19}NO_4$ 205.26		Viscous liquid $[\alpha]_D^{20}+29.5°$	Synthetic. Shows 86% of activity of pantothenic acid in the chick test[2]
Pantethein (*N*-pantothenyl-β-aminoethanethiol)	$C_{11}H_{22}N_2O_4S$ 278.37		Amorphous powder soluble in water	Growth factor for *Lactobacillus bulgaricus*
Coenzyme A (CoA)	$C_{21}H_{36}N_7O_{16}P_3S$ 767.54	See page 345	Colourless powder soluble in water	Widely distributed in micro-organisms, plants and animals. See also text and page 345

intestine, though it is not known whether the body makes use of this source. The pantothenic acid content of whole blood is ca. 0.2–2 mg/l (see page 611), that of the spinal fluid about the same (see page 639). Urinary excretion of the vitamin amounts to 0.76–4.1 mg/l[3] (see also page 676). Four hours after giving a test dose of the vitamin there is a sharp rise in the blood and urine levels[4]. In blood and spinal fluid pantothenic acid is in the conjugated form, whereas in the urine it is in the free form[3]. The excretion in the faeces is very variable and depends on the nature of the diet.

Coenzyme A is not present in the circulating blood and appears to pass the cell membrane only with difficulty. It is probably formed within the cell when required. Coenzyme A occurs mainly in the following organs (in order of decreasing concentration): liver, adrenals, kidneys, brain, heart, testes. The pantothenic acid content of the human liver amounts to 28 mg, mainly as coenzyme A[9].

Function

The importance of pantothenic acid in metabolism is explained by its presence in coenzyme A. In the form of acetyl-coenzyme A ('active acetic acid') it is responsible for transport of two-carbon and other acyl groups (see page 345). Coenzyme A is involved in the following reactions: formation of citrate from oxalacetate and acetate (pages 390 and 424), oxidation of pyruvate (page 391), oxidation of α-ketoglutarate (page 390), oxidation and synthesis of fatty acids (pages 391 and 424), synthesis of triglycerides (page 426), phospholipids (page 425) and cholesterol (page 426), acetylation of amines (page 444), choline (page 434) and glucosamine (see page 424). Pantothenic acid plays an important part in the activity of the adrenal cortex[10] since the corticosteroids are formed from the cholesterol synthesized with the participation of coenzyme A (page 429).

Requirements and deficiency symptoms

The human pantothenic acid requirement is unknown, but in children and adults it is probably met by an intake of 5–10 mg per day[11], an amount normally present in the diet. The requirements of children are lower in accordance with their smaller intake of calories[12].

Pantothenic acid is present in almost all vegetables, cereals and animal foods. Good sources of the vitamin are yeast, liver, kidneys, heart (see pages 499–515) and particularly the jelly queen bees are fed on (royal jelly), containing 110–320 μg/g[13].

Pantothenic acid is so widely distributed in foods that deficiency is practically unknown in man. Deficiency symptoms in animals are degeneration of the neuromuscular structures and adrenal insufficiency, and death may ensue. An experimental deficiency has been produced in man by means of a diet low in the vitamin together with doses of the antagonist Ω-methylpantothenic acid[14], with the following symptoms: mild fatigue, headache, sleeplessness, nausea, epigastric pain, paraesthesia of the limbs, muscle spasms and co-ordination disturbances; other signs were absence of the eosinophile reaction of the blood to ACTH and an increased sensitivity to insulin. The pantothenic acid deficiency was evidenced by the reduced acetylating capacity of the blood after administration of sulphanilamide or para-aminobenzoic acid.

Treatment

The so-called 'burning feet' syndrome has been treated with pantothenic acid[15], though it is not certain that this syndrome is solely due to pantothenic acid deficiency. Pantothenic acid has been reported to be effective against the neurotoxicity of streptomycin[16]; its value in the treatment of emotional disturbances[17], diabetic neuropathy[17], skin diseases[17] and paralytic ileus[18] remains uncertain.

References ('Pantothenic acid', pages 487-

[1] Sebrell and Harris (Eds.), *The Vitam* York, 1954, page 589; Chow, B. F., in *Nutrition*, vol. 2, Academic Press, New Yo in Wohl and Goodhart (Eds.), *Moder* 3rd ed., Lea & Febiger, Philadelphia, 19
[2] Hegsted, D. M., *Proc. Soc. exp. Biol. (N.* 1
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[4] Gyttenberg, F., *Chemisch-physikalische Vit* Enke, Stuttgart, 1965, page 231.
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Ascorbic acid[1–3] (for references see p

Chemistry

Compound	Formula a mol. wt.
L-Ascorbic acid (vitamin C)	$C_6H_8O_6$ 176.13
Dehydroascorbic acid	$C_6H_6O_6$ 174.11
2-Keto-L-gulonic acid	$C_6H_{10}O_7$ 194.14

Assay

Biological[4]. In guinea-pigs either by structure or by means of the preventi

Chemical[5]. Polarographically; by ability of ascorbic acid to reduce 2,6-*N*-bromosuccinimide (dehydroascorb forehand with, for instance, H_2S or cally using the 2,4-dinitrophenylhyd acid and 2,3-diketo-L-gulonic acid, w concentrated sulphuric acid (ascorbic hand with, for instance, bromine or ac best extracted from biological materia solution. Ascorbic acid and dehydroa from one another and from other sul graphy.

Unit

No international unit; by weight. T (= 50 μg L-ascorbic acid) is now obsc

Biogenesis[6]

Ascorbic acid is synthesized by hi except the primates, guinea-pigs, the *tafer*) and the Indian fruit bat (*Pteropus* ently not formed by micro-organisms, sary for their growth. Biogenesis take

rties	Occurrence and activity
stable nd	Widely distributed in plants and animals. Growth factor for yeasts, many other micro-organisms and all higher animals; component of coenzyme A
white to	
	Synthetic. Shows 86% of activity of pantothenic acid in the chick test[2]
owder er	Growth factor for *Lactobacillus bulgaricus*
wder er	Widely distributed in micro-organisms, plants and animals. See also text and page 345

cy symptoms

cid requirement is unknown, but in ably met by an intake of 5–10 mg per esent in the diet. The requirements of nce with their smaller intake of calo-

in almost all vegetables, cereals and f the vitamin are yeast, liver, kidneys, l particularly the jelly queen bees are g 110–320 μg/g[13].
ly distributed in foods that deficiency ı. Deficiency symptoms in animals are ıscular structures and adrenal insuffi- An experimental deficiency has been of a diet low in the vitamin together 2-methylpantothenic acid[14], with the igue, headache, sleeplessness, nausea, of the limbs, muscle spasms and co- er signs were absence of the eosino- ACTH and an increased sensitivity to ! deficiency was evidenced by the re- the blood after administration of sul- nzoic acid.

et' syndrome has been treated with t is not certain that this syndrome is deficiency. Pantothenic acid has been t the neurotoxicity of streptomycin[16]; notional disturbances[17], diabetic neu- d paralytic ileus[18] remains uncertain.

References ('Pantothenic acid', pages 487–488)

1 Sebrell and Harris (Eds.), *The Vitamins*, vol. 2, Academic Press, New York, 1954, page 589; Crow, B.F., in Beaton and McHenry (Eds.), *Nutrition*, vol. 2, Academic Press, New York, 1964, page 207; Vilter, R.W., in Wohl and Goodhart (Eds.), *Modern Nutrition in Health and Disease*, 3rd ed., Lea & Febiger, Philadelphia, 1964, page 395.
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12 Szőrády, I., *Mschr. Kinderheilk.*, 111, 10 (1963).
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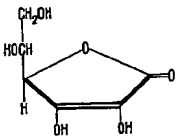
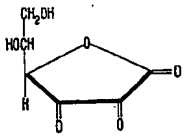
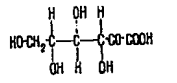
18 Editorial, *Brit. med. J.*, 2, 634 (1963).

Ascorbic acid[1–3] (for references see page 491)

Chemistry

Compound	Formula and mol. wt.	Structure	Physical properties	Occurrence and activity
L-Ascorbic acid (vitamin C)	$C_6H_8O_6$ 176.13		White crystals with an acid taste, soluble in water, rather insoluble in ethanol, sensitive to light, atmospheric oxygen and some heavy metals; strong reducing action M.p. 192 °C. $[\alpha]_D$ +23°	Probably present in all higher plants, particularly cabbage, citrus fruits, hawthorn berries; in small amounts in animal tissues. Antiscorbutic activity
Dehydroascorbic acid	$C_6H_6O_6$ 174.11		White crystals, soluble in water; readily hydrolysed to 2,3-diketo-L-gulonic acid M.p. 225 °C	Present with ascorbic acid in plants. Antiscorbutic activity
2-Keto-L-gulonic acid	$C_6H_{10}O_7$ 194.14		White crystals M.p. 171 °C	Precursor of ascorbic acid. No antiscorbutic activity

Assay

Biological[4]. In guinea-pigs either by histological study of tooth structure or by means of the preventive or curative growth test.

Chemical[5]. Polarographically; by mass analysis based on the ability of ascorbic acid to reduce 2,6-dichlorphenolindophenol or *N*-bromosuccinimide (dehydroascorbic acid must be reduced beforehand with, for instance, H_2S or homocysteine); photometrically using the 2,4-dinitrophenylhydrazones of dehydroascorbic acid and 2,3-diketo-L-gulonic acid, which give a red solution in concentrated sulphuric acid (ascorbic acid must be oxidized beforehand with, for instance, bromine or active charcoal). The vitamin is best extracted from biological materials with metaphosphoric acid solution. Ascorbic acid and dehydroascorbic acid can be separated from one another and from other substances by paper chromatography.

Unit

No international unit; by weight. The former International Unit (= 50 μg L-ascorbic acid) is now obsolete.

Biogenesis[6]

Ascorbic acid is synthesized by higher plants and all animals except the primates, guinea-pigs, the red-vented bubul (*Pycnonotus cafer*) and the Indian fruit bat (*Pteropus medius*). The vitamin is apparently not formed by micro-organisms, and is presumably not necessary for their growth. Biogenesis takes place in the following steps (there is some doubt as to whether these are valid for plants):

D-Glucose ⟶ D-Glucuronic acid ⟶ D-Glucuronolactone ⟶ L-Gulonolactone (L-gulonolactone oxidase) ⟶ 2-Keto-L-gulonic acid ⟶ L-Ascorbic acid

In most animals ascorbic acid is synthesized in the liver, but in birds, reptiles and amphibia in the kidneys. Animals that do not form ascorbic acid do not possess the enzyme L-gulonolactone oxidase.

Intake and excretion

In the USA the daily intake of ascorbic acid is about 120 mg, half of which arises from citrus fruits and tomatoes[7]. In the gastrointestinal tract ascorbic acid is absorbed in the same way as glucose and other carbohydrates[9]. Even with high intakes of ascorbic acid less than 10 mg of the vitamin is excreted per day in the faeces[8]. Equilibrium between ascorbic acid absorbed from the diet and that present in the tissues is reached in less than 4 hours. Ascorbic acid probably passes the cell membranes in the more lipid-soluble dehydroascorbic acid form, which is then again reduced to ascorbic acid in the cell[9,10]. When the tissues are saturated with ascorbic acid the overall body concentration of the vitamin is 50 mg per kg[11]. The ascorbic acid contents (mg/kg) of various tissues in human adults are as follows[12]: brain 150, pituitary 150, lens of the

eye 250, adrenals 400, pancreas 150, liver 150, kidneys 50, heart muscle 50. Tissue levels of ascorbic acid are highest at birth and greatly reduced in old age[12]. The saturation level in leucocytes is 270–300 mg ascorbic acid per kg[11]; high doses of ascorbic acid have been reported to increase this concentration up to 600 mg per kg[13]. The whole blood and plasma levels (see page 611) depend on the degree of tissue saturation and on the dietary intake. High doses of ascorbic acid cause the plasma level to rise rapidly, possibly to 40 mg per litre; the normal plasma level is below 14 mg per litre, since at this concentration the threshold value for the kidneys is reached with a consequent steep rise in the ascorbic acid clearance[3,11]. In the plasma about 20% of the total ascorbic acid is in the form of dehydroascorbic acid[14] (page 611). Ascorbic acid also occurs in the aqueous humour of the eye (50–295 mg/l[15]), in gastric juice (see page 650) and in the synovial fluid (see page 642).

Dependence of ascorbic acid levels in serum and leucocytes on intake[2,3,11]

Daily intake (mg)	Serum (mg/l)	Leucocytes (mg/kg)	Tissue saturation (%)	Percentage of a test dose in the urine*
<10	<2	<120	0–40	<5
10–20	~2	~120	~40	<15
30–100	4–10	150–200	>50	20–60
>100	10–14	270–300	→100	60–80

* The usual loading tests consist either of giving 10 mg ascorbic acid orally per kg body weight followed by determination of ascorbic acid in the 24-hour urine, or of giving 100 mg ascorbic acid intravenously followed by determination of ascorbic acid in the 3-hour urine.

With an ascorbic acid concentration of 20 mg per kg body weight the daily turnover is about 1 mg per kg, corresponding to a half-life of 16 days[16]. In urine ascorbic acid appears mainly unchanged (see page 676), but part is hydrolysed to diketogulonic acid and appears finally as oxalic acid[3]. Other metabolites are L-xylonic and L-lyxonic acids, which arise by decarboxylation of ascorbic acid[17]. Radioactive CO_2 has been detected in expired air after giving doses of tagged ascorbic acid[16]. The ascorbic acid content of breast milk (page 689) depends largely on the dietary intake.

Function

Ascorbic acid and dehydroascorbic acid form a redox system with 'semidehydroascorbic acid' as highly reactive intermediate. The latter arises by the loss of a single electron by ascorbic acid or by the acceptance of a single electron by dehydroascorbic acid[10]. Almost all metabolic processes the disturbance of which gives rise to scurvy involve reactions in which ascorbic acid is oxidized. Of particular importance are the hydroxylation reactions dependent on ascorbic acid which require molecular oxygen.

The disturbances in the formation of connective tissue that appear in ascorbic acid deficiency are a result of failure of the ascorbic acid-dependent hydroxylation of proline to hydroxyproline, a component of collagen. The formation and maintenance of collagen is dependent on a normal ascorbic acid level[19]. Synthesis of collagen in human skin-tissue cultures is promoted by ascorbic acid[20]. In guinea-pigs with ascorbic acid deficiency the formation of hydroxyproline in granulation tissue commences only after ascorbic acid has been administered[21].

Another hydroxylation reaction dependent on ascorbic acid is the hydroxylation of the side chain of dopamine to noradrenaline; the enzyme catalysing this reaction has been found in the microsomes of beef adrenal cortex[22]. Ascorbic acid is probably involved also in the hydroxylations occurring in steroid synthesis in the adrenals, but little is known of this role[22,23].

Ascorbic acid is also concerned in tyrosine metabolism as reducing agent, although here it does not participate in a hydroxylation reaction. Its probable effect is that of protecting the enzyme para-hydroxyphenylpyruvic acid hydroxylase from inhibition by its substrate[24]. Ascorbic acid also plays a role as electron donor in the conversion of folic acid into tetrahydrofolic acid. This connection between ascorbic acid and tetrahydrofolic acid may be responsible for the appearance of macrocytic anaemia in scurvy[10]. The hypochromic anaemia of scurvy, on the other hand, is more likely to be due to an effect of ascorbic acid on iron metabolism, since the vitamin is necessary for the incorporation of iron into ferritin[25].

In contrast to the reactions dependent on ascorbic acid, the hydroxylation of tryptophan to 5-hydroxytryptophan, the precursor of serotonin, is mediated by dehydroascorbic acid, which is thereby reduced to ascorbic acid. The enzyme catalysing this reaction is dependent on copper ions for its activity and occurs mainly in the tissues of the small intestine[26]. The regeneration of ascorbic acid from dehydroascorbic acid in the tissues plays an important metabolic role. It takes place with the formation of semidehydroascorbic acid from ascorbic acid and dehydroascorbic acid, whereby an enzyme system present in the animal cell transfers electrons from $NADH_2$ to semidehydroascorbic acid and so regenerates ascorbic acid[10].

In the animal organism ascorbic acid seems to have a protective action against deficiencies of other vitamins (thiamine, riboflavin, pantothenic acid, biotin, folic acid, vitamin E, vitamin A); the relationships between these vitamins and ascorbic acid are, however, obscure[27].

Requirements and deficiency symptoms

The minimum intake of ascorbic acid required to prevent scurvy in infants is about 10 mg per day, in adults a little under 10 mg per day[28]. A daily intake of 30–40 mg results in moderate saturation of the tissues, one of 60–100 mg in almost complete saturation[28]. The recommended daily intakes of ascorbic acid in various countries are as follows: England[32]: infants 15 mg, children 20–30 mg, adults 30 mg; USA[29]: infants 35 mg, children 40–80 mg, adults 60 mg; Western Germany[30]: infants 30–35 mg, children 40–50 mg, adults 75 mg; Canada[31]: children 20–30 mg, adults 30 mg; Holland[33]: children 35–75 mg, adults 50 mg; Japan[34]: children 30–90 mg, adults 60–65 mg. For the recommendations of the Food and Nutrition Board (USA)[29] for pregnant and lactating women see the table on page 494. Workers in very cold climates should have an intake of 150–250 mg per day[35].

Good sources of ascorbic acid are cabbage, spinach, paprikas, citrus fruits, tomatoes, strawberries, red currants and liver (see pages 499–515). In vegetables the ascorbic acid falls rapidly during withering. Potatoes are an important source of ascorbic acid but the concentration decreases by up to 80% in winter storage. Fresh cow's milk contains up to 25 mg per litre; this is markedly reduced by pasteurization or boiling.

The most important symptoms of ascorbic acid deficiency are a marked tendency to bleeding with the appearance of extensive patches of haemorrhage under the skin and in the gums, muscles, fatty tissues and internal organs. Other symptoms are impairment of connective tissue formation with changes in bone structure and growth, defective tooth formation and fissuring and roughening of the skin; there are also often disturbances of iron absorption and anaemia. In infants ascorbic acid deficiency (Moeller-Barlow disease) is manifested mainly in the bones, which show a zone of destroyed bone extending over the margin of the metaphysis into the soft tissues, as well as by subperiostal bleeding, particularly in the metaphyseal zones of the long bones.

On a diet free of ascorbic acid the ascorbic acid content of the plasma falls after 40 days to less than 1 mg per litre, while after 120 days that of the leucocytes is almost zero[2]. At this time there is also enlargement and keratosis of the hair follicles, which in the succeeding 40 days gradually develop haemorrhages and the characteristic signs of scurvy; changes in the gingiva appear after 180 days[36].

An inadequate intake of ascorbic acid is best recognized by the lowered plasma level, whereas a severe deficiency of the vitamin is characterized by the low ascorbic acid concentration of the leucocytes (< 120 mg/kg; see the table above). Various tests are available for measuring the extent to which ascorbic acid is provided by the diet; they depend on the degree of tissue saturation effected by a test dose of the vitamin as indicated by the plasma level and urinary excretion.

Treatment

In infants, doses of 20 mg ascorbic acid per day, for instance in the form of orange juice, are ample to prevent the appearance of scurvy; in infants with scurvy 25 mg should be given 4 times a day, in adults with the disease 100 mg 5 or 6 times a day[28]. In patients who have undergone extensive surgery 150–300 mg ascorbic acid per day is sufficient to produce adequate saturation of the tissues[37]. The reducing properties of ascorbic acid can be utilized in the treatment of methaemoglobinaemia and to promote the absorption of orally administered iron[38]. Russian workers have reported success in the treatment of coronary diseases with ascorbic acid[39].

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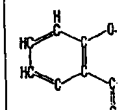
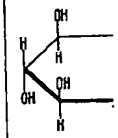
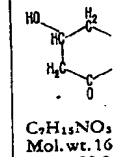
Substances with vitamin-like

Compound	Formula physic
Bioflavonoids[2] (vitamin P group, citrin)	Structure of  Substances and eriodict this group
Mesoinositol[4] (myoinositol)	 $C_6H_{13}O_6$ Mol. wt. 18(M. p. 225–2
Carnitine[6] (β-hydroxy-γ-butyro-betaine, vitamin B_T)	 $C_7H_{15}NO_3$ Mol. wt. 16 $[\alpha]_D$ −20.9

* According to Bersin[1] these are su the form of enzymes.

ns dependent on ascorbic acid, the
1 to 5-hydroxytryptophan, the pre-
ed by dehydroascorbic acid, which is
cid. The enzyme catalysing this reac-
ons for its activity and occurs mainly
stine[26]. The regeneration of ascorbic
cid in the tissues plays an important
with the formation of semidehydro-
id and dehydroascorbic acid, whereby
he animal cell transfers electrons from
rbic acid and so regenerates ascorbic

corbic acid seems to have a protective
other vitamins (thiamine, riboflavin,
ic acid, vitamin E, vitamin A); the
vitamins and ascorbic acid are, how-

cy symptoms

corbic acid required to prevent scurvy
day, in adults a little under 10 mg per
0 mg results in moderate saturation of
in almost complete saturation[28]. The
of ascorbic acid in various countries
infants 15 mg, children 20–30 mg,
its 35 mg, children 40–80 mg, adults
infants 30–35 mg, children 40–50 mg,
ildren 20–30 mg, adults 30 mg; Hol-
dults 50 mg; Japan[34]: children 30–90
ie recommendations of the Food and
or pregnant and lactating women see
ers in very cold climates should have
day[35].
acid are cabbage, spinach, paprikas,
wberries, red currants and liver (see
s the ascorbic acid falls rapidly during
mportant source of ascorbic acid but
oy up to 80% in winter storage. Fresh
mg per litre; this is markedly reduced

toms of ascorbic acid deficiency are a
ng with the appearance of extensive
er the skin and in the gums, muscles,
ans. Other symptoms are impairment
on with changes in bone structure and
mation and fissuring and roughening
en disturbances of iron absorption and
c acid deficiency (MOELLER-BARLOW
y in the bones, which show a zone of
ver the margin of the metaphysis into
subperiostal bleeding, particularly in
long bones.
acid the ascorbic acid content of the
less than 1 mg per litre, while after
es is almost zero[2]. At this time there is
sis of the hair follicles, which in the
develop haemorrhages and the charac-
nges in the gingiva appear after 180

scorbic acid is best recognized by the
as a severe deficiency of the vitamin is
orbic acid concentration of the leuco-
table above). Various tests are avail-
t to which ascorbic acid is provided by
degree of tissue saturation effected by a
dicated by the plasma level and urinary

; ascorbic acid per day, for instance in
e ample to prevent the appearance of
y 25 mg should be given 4 times a day,
0 mg 5 or 6 times a day[28]. In patients
ive surgery 150–300 mg ascorbic acid
ce adequate saturation of the tissues[37].
scorbic acid can be utilized in the treat-
nia and to promote the absorption of
Russian workers have reported success
diseases with ascorbic acid[39].

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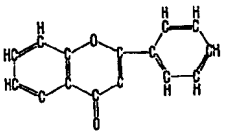
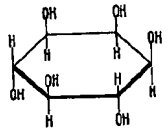
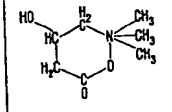
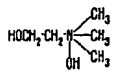
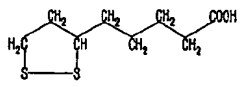
Substances with vitamin-like action (vitaminoids)*

Compound	Formula, mol. wt. and physical properties	Occurrence	Function	Requirements and deficiency symptoms
Bioflavonoids[2] (vitamin P group, citrin)	Structure of the flavones:  Substances like hesperidine and eriodictiol also belong to this group	Widely distributed in plants, particularly in fruits (e.g., lemons and black currants)	Biological function obscure. Views on pharmacological activity vary. Substances increasing capillary resistance probably possess antihistamine and antihyaluronidase activity[3]	
Mesoinositol[4] (myoinositol)	 $C_6H_{12}O_6$ Mol. wt. 180.16 M.p. 225–227 °C	Probable component of all living cells. Component of phospholipids in leaves, seeds and animal tissues (particularly heart, brain and skeletal muscle); present as hexaphosphate (phytic acid) in plants	In the phospholipid form involved in cation transport through the cell membranes, in stimulation of nerves and in metabolism of the mitochondria[5]	Growth factor for yeasts and many kinds of animal cells in tissue culture. Synthesized in the animal organism. Significance in human nutrition obscure. Intake about 1 g per day
Carnitine[6] (β-hydroxy-γ-butyro-betaine, vitamin B_T)	 $C_7H_{15}NO_3$ Mol. wt. 161.20 $[\alpha]_D$ −20.9°	In all animal tissues (skeletal muscle 1, heart 0.6, kidneys 0.4, liver 0.3 mg/gramme dry substance); small amounts in blood, milk, plants and micro-organisms	Involved in intracellular fat metabolism in the form of acylcarnitines by (a) transporting acetyl-coenzyme A and acetoacetyl-coenzyme A from the mitochondria to the site of synthesis of long-chain fatty acids outside the mitochondria[7]; (b) transporting activated long-chain acyl groups from the cytoplasm to the mitochondria, where long-chain fatty acids are oxidized[8]	Growth factor for some insects, for instance mealworm, *Tenebrio molitor* and some bacterial species. Vertebrates can synthesize carnitine

* According to BERSIN[1] these are substances that must be regarded as essential dietary components for many living organisms but that do not function in the form of enzymes.

Compound	Formula, mol. wt. and physical properties	Occurrence	Function	Requirements and deficiency symptoms
Choline[9] (β-hydroxyethyltrimethylammonium hydroxide)	 $C_5H_{13}NO_2$ Mol. wt. 121.18 M.p. 180 °C (decomp.)	Component of lecithin, plasmalogens, sphingomyelin and acylcholines. Widely distributed in plants and animals (egg-yolk 17, meat 6, cereals 1 mg per gramme). See also pages 394 and 434	Methyl-group donor, replaceable by other sources of labile methyl groups or when synthesized from the latter in the body. Involved in transport of fatty acids from the liver to peripheral fat stores	Growth factor for various micro-organisms, also for rats, chickens and turkeys. Choline deficiency in animals results in injury to the liver and kidneys. Significance in human nutrition obscure. Intake about 0.5–0.9 g per day, an adequate amount in the light of the known requirement of 0.1–0.15 g per 100 g of food[10]
α-Lipoic acid[11] (thioctic acid)	 $C_8H_{14}O_2S_2$ Mol. wt. 206.33 M.p. 48 °C	In small amounts in vegetable and animal tissues, particularly yeasts and liver	Involved in the oxidative decarboxylation of pyruvic and α-ketoglutaric acids (see pages 390 and 391)	Growth factor for certain bacterial and protozoal species. Probably of no importance in nutrition of higher animals
Essential fatty acids[12] (vitamin F)	Linoleic and arachidonic acids (see page 370) Unsaturated fatty acids and related compounds such as the corresponding alcohols not synthesized by the body in adequate quantity but necessary for metabolism and growth	In the diet, particularly linoleic acid (plant oils, animal fats) and arachidonic acid (animal fats). Also active are linoleic acid precursors such as linoleyl alcohol and cis-2-octenic acid[13]; linolenic acid is almost inactive. Arachidonic acid is synthesized in the animal body from linoleic acid	Involved in formation of the cell membranes and possibly transport of fatty acids. Structurally essential components of phospholipids and precursors of prostaglandins. Play a role in metabolism of the mitochondria. Many polyene fatty acids such as linoleic and arachidonic acids but also others with no effect on growth are capable of lowering the serum cholesterol level	Deficiency symptoms: in young rats cessation of growth and eczema; in infants eczema[14]. The increased water intake in rats with deficiency can be utilized to measure activity[15]. In deficiency the triene acid content of the serum is increased (formation of 5,8,11- and 7,10,13-eicosatrienic acids), the tetraene acid content (arachidonic acid) lowered[16,17]. The minimum essential fatty acid requirement in man is about 1–2% of the calorie intake[16,18], or 1.2–2.4 g linoleic acid per 1000 kcal dietary intake. Optimal requirement in infancy: 4% of the calorie intake in the form of linoleic acid[19]. The tocopherol requirement rises with increasing intake of essential fatty acids (see page 466)

* See footnote, page 491.

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A *nutritional standard* is a statei
nutrients (usually the average daily
for a person representative of the
which the standard applies[7]. Term
and nutritional allowance are also
regarded as synonymous. The re
minimum, average or desirable. Si
define an individual's optimum rec
uent, other methods of evaluating
come necessary. In USA, the reco

Recommended daily allowances of calor

Age
0–3 months **
4–6 months **
7–12 months
1 year
2 years
3 years
4–6 years
7–9 years
10–12 years
13–15 (boys)
(girls)
16–19 (boys)
(girls)
Adults (men)
(women)

* The amounts given do not cover infections, malabsorption, metabolic conditions, etc. They are also applic calories and all other nutrients are ful

** For infants from 0 to 6 months, well-nourished mother is the best way for these vitamins.

Daily protein requirements (FAO/W

Age
Infants†
0–3 months
3–6 months
6–9 months
9–12 months
Juveniles
1–3 years
4–6 years
7–9 years
10–12 years
13–15 years
16–19 years
Adults††

* The composition of the Reference proteins of eggs, milk and meat all h intake of proteins of lower biologic increased.

** This range is based on the expected upper level is likely to cover the requ and can thus be regarded as a *practica*

† In terms of either breast milk or co

†† During the second and third trimest be added, during lactation 15 g per d

Calories †

The body requires food energy f of body tissues, physical activities tenance of thermal balance. The e levels of activity are given in the t

inoids)*

n	Requirements and deficiency symptoms
lonor, re- er sources groups or ed from body. In- sport of the liver stores	Growth factor for various micro-organisms, also for rats, chickens and turkeys. Choline deficiency in animals results in injury to the liver and kidneys. Significance in human nutrition obscure. Intake about 0.5–0.9 g per day, an adequate amount in the light of the known requirement of 0.1–0.15 g per 100 g of food[10]
oxidative of pyru- oglutaric 390 and	Growth factor for certain bacterial and protozoal species. Probably of no importance in nutrition of higher animals
ation of nes and of fatty y essen- of phos- ecursors . Play a n of the ay poly- as lino- ic acids vith no are ca- the se- vel	Deficiency symptoms: in young rats cessation of growth and eczema; in infants eczema[14]. The increased water intake in rats with deficiency can be utilized to measure activity[15]. In deficiency the triene acid content of the serum is increased (formation of 5,8,11- and 7,10,13-eicosatrienic acids), the tetraene acid content (arachidonic acid) lowered[16,17]. The minimum essential fatty acid requirement in man is about 1–2% of the calorie intake[16,18], or 1.2–2.4 g linoleic acid per 1000 kcal dietary intake. Optimal requirement in infancy: 4% of the calorie intake in the form of linoleic acid[19]. The tocopherol requirement rises with increasing intake of essential fatty acids (see page 466)

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p, *Wld Hlth Org. techn. Rep. Ser.*, No. 362

:0, 1 (1962).
:r., 41, 1 (1961).
7 (1962).
:1958).
iforsch., 25, 62 (1953).
.., 178, 930 (1961).
, 24, 1 (1966).
r., 14, 70 (1964).
)58).

(For references see page 497)

A *nutritional standard* is a statement of the amounts of certain nutrients (usually the average daily amounts) regarded as necessary for a person representative of the category of the population to which the standard applies[1]. Terms such as nutritional requirement and nutritional allowance are also used but must not always be regarded as synonymous. The resulting diets are referred to as minimum, average or desirable. Since it is at present impossible to define an individual's optimum requirement of any dietary constituent, other methods of evaluating nutritional standards have become necessary. In USA, the recommended dietary *allowances*[2] are designed to provide an adequate diet to cover individual variations among most normal persons of the population. Comparisons between the various standards laid down by national and international bodies are possible only when the purpose of the standards compared is known. In the tables below and on pages 494–495 the standards of the FAO[3] and the Joint FAO/WHO Expert Groups[4–6] and of the Food and Nutrition Board (USA) are given in detail. For other national standards (British, Canadian, Indian, South African, Australian, Dutch, Norwegian, Central American, Russian) see YOUNG[7]. A new UK standard has recently been published[38].

Recommended daily allowances of calories (FAO[3]) *and of vitamin A (retinol), thiamine, riboflavin and niacin** (Joint FAO/WHO Expert Group[6])

Age	Calories per day	Retinol† (mg)	Thiamine (mg)	Riboflavin (mg)	Niacin equivalents††
0–3 months**	120 per kg	–	–	–	–
4–6 months**	110 per kg	–	–	–	–
7–12 months	1000	300	0.4	0.6	6.6
1 year	1150	250	0.5	0.6	7.6
2 years	1300	250	0.5	0.7	8.6
3 years	1450	250	0.6	0.8	9.6
4–6 years	1700	300	0.7	0.9	11.2
7–9 years	2100	400	0.8	1.2	13.9
10–12 years	2500	575	1.0	1.4	16.5
13–15 (boys)	3100	725	1.2	1.7	20.4
(girls)	2600	725	1.0	1.4	17.2
16–19 (boys)	3600	750	1.4	2.0	23.8
(girls)	2400	750	1.0	1.3	15.8
Adults (men)	3200	750	1.3	1.8	21.1
(women)	2300	750	0.9	1.3	15.2

* The amounts given do not cover abnormal needs such as those due to infections, malabsorption, metabolic disturbances, extreme environmental conditions, etc. They are also applicable only when the requirements for calories and all other nutrients are fully met.

** For infants from 0 to 6 months, it is accepted that breast feeding by a well-nourished mother is the best way to satisfy the nutritional requirements for these vitamins.

† For diets containing both carotene and retinol, adjustment must be made as follows: recommended intake of mixed vitamin A-active compounds = $\frac{\text{recommended intake of retinol}}{0.167\,k + (1-k)}$, where $k = \frac{\beta\text{-carotene } (\mu g)}{\beta\text{-carotene } (\mu g) + \text{retinol } (\mu g)}$

†† A niacin equivalent is 1 mg niacin or 60 mg L-tryptophan.

Daily protein requirements (FAO/WHO Expert Group[4])

Age	Grammes Reference Protein* per kilogramme body weight	
	Average	Range**
Infants†		
0–3 months	2.3	–
3–6 months	1.8	–
6–9 months	1.5	–
9–12 months	1.2	–
Juveniles		
1–3 years	0.88	0.70–1.06
4–6 years	0.81	0.65–0.97
7–9 years	0.77	0.62–0.92
10–12 years	0.72	0.58–0.86
13–15 years	0.70	0.56–0.84
16–19 years	0.64	0.51–0.77
Adults††	0.59	0.47–0.71

* The composition of the Reference Protein is given on page 516. The proteins of eggs, milk and meat all have the same biological value. The intake of proteins of lower biological value must be correspondingly increased.

** This range is based on the expected range of individual variation; the upper level is likely to cover the requirements of 95% of the population and can thus be regarded as a *practical allowance*.

† In terms of either breast milk or cow's milk protein.

†† During the second and third trimesters of pregnancy 6 g per day should be added, during lactation 15 g per day.

Desirable daily calcium allowances (FAO/WHO Expert Group[5])

Age	mg/day
0–12 months*	500– 600
1–9 years	400– 500
10–15 years	600– 700
16–19 years	500– 600
Adults	400– 500
Pregnancy, 3rd trimester	1000–1200
Lactation	1000–1200

* For infants not being breast-fed; the calcium requirement of an infant being breast-fed by a normally lactating mother is met by the breast milk.

Calories†

The body requires food energy for resting metabolism, synthesis of body tissues, physical activities, excretory processes and maintenance of thermal balance. The energy requirements at different levels of activity are given in the tables on page 495.

The calorie allowances of the Food and Nutrition Board (USA), like those of the FAO, are based on the concept of a 'reference' subject. In the case of the former this is a man aged 22 weighing 70 kg or a woman aged 22 weighing 58 kg with a 'light' level of physical activity in a mean environmental temperature of 20 °C. It is assumed that weight gains after this age are likely to be fat. Since it is difficult to estimate the degree of reduction in physical activity associated with advancing age the calorie allowances (see table on page 494) were obtained by making a reduction of 5% between the ages of 22 and 35, of 3% per decade between 35 and 55, and of 5% per decade between 55 and 75. A further reduction of 7% is recommended for persons aged 75 and over. The upper left-hand table on page 495 gives the adjustment for persons of other than the ideal weight.

Increased physical activity also requires an increase in the calorie intake, though more than an additional 1500 kcal over the allowances recommended in the table is rarely necessary. A lower level of physical activity (e.g., in persons with a sedentary occupation) calls for a lower calorie intake.

An environmental temperature lower than 20°C necessitates little if any increase in calorie intake provided that adequate but not

† 1 $kcal_{15} = 4.1855$ kJ (see page 213).

Nutritional Standards

(For references see page 497)

*Recommended daily dietary allowances** (Food and Nutrition Board, USA[2])

	Age** (years)	Weight (kg)	Weight (lb)	Height cm	Height (in)	Calories (kcal)	Protein (g)	Minerals: Calcium (g)	Phosphorus (g)	Iodine (μg)	Iron (mg)	Magnesium (mg)
Infants	0–1/6	4	9	55	22	kg×120	kg×2.2***	0.4	0.2	25	6	40
	1/6–1/2	7	15	63	25	kg×110	kg×2.0***	0.5	0.4	40	10	60
	1/2–1	9	20	72	28	kg×100	kg×1.8***	0.6	0.5	45	15	70
Children	1–2	12	26	81	32	1100	25	0.7	0.7	55	15	100
	2–3	14	31	91	36	1250	25	0.8	0.8	60	15	150
	3–4	16	35	100	39	1400	30	0.8	0.8	70	10	200
	4–6	19	42	110	43	1600	30	0.8	0.8	80	10	200
	6–8	23	51	121	48	2000	35	0.9	0.9	100	10	250
	8–10	28	62	131	52	2200	40	1.0	1.0	110	10	250
Males	10–12	35	77	140	55	2500	45	1.2	1.2	125	10	300
	12–14	43	95	151	59	2700	50	1.4	1.4	135	18	350
	14–18	59	130	170	67	3000	60	1.4	1.4	150	18	400
	18–22	67	147	175	69	2800	60	0.8	0.8	140	10	400
	22–35	70	154	175	69	2800	65	0.8	0.8	140	10	350
	35–55	70	154	173	68	2600	65	0.8	0.8	125	10	350
	55–75+	70	154	171	67	2400	65	0.8	0.8	110	10	350
Females	10–12	35	77	142	56	2250	50	1.2	1.2	110	18	300
	12–14	44	97	154	61	2300	50	1.3	1.3	115	18	350
	14–16	52	114	157	62	2400	55	1.3	1.3	120	18	350
	16–18	54	119	160	63	2300	55	1.3	1.3	115	18	350
	18–22	58	128	163	64	2000	55	0.8	0.8	100	18	350
	22–35	58	128	163	64	2000	55	0.8	0.8	100	18	300
	35–55	58	128	160	63	1850	55	0.8	0.8	90	18	300
	35–75+	58	128	157	62	1700	55	0.8	0.8	80	10	300
Pregnancy						+ 200	65	+0.4	+0.4	125	18	450
Lactation						+ 1000	75	+0.5	+0.5	150	18	450

	Age** (years)	Fat-soluble vitamins: Vitamin A activity (IU)	Vitamin D (IU)	Vitamin E activity (IU)	Water-soluble vitamins: Ascorbic acid (mg)	Folacin† (mg)	Niacin†† (mEq)	Riboflavin (mg)	Thiamine (mg)	Vitamin B_6 (mg)	Vitamin B_{12} (μg)
Infants	0–1/6	1500	400	5	35	0.05	5	0.4	0.2	0.2	1.0
	1/6–1/2	1500	400	5	35	0.05	7	0.5	0.4	0.3	1.5
	1/2–1	1500	400	5	35	0.1	8	0.6	0.5	0.4	2.0
Children	1–2	2000	400	10	40	0.1	8	0.6	0.6	0.5	2.0
	2–3	2000	400	10	40	0.2	8	0.7	0.6	0.6	2.5
	3–4	2500	400	10	40	0.2	9	0.8	0.7	0.7	3
	4–6	2500	400	10	40	0.2	11	0.9	0.8	0.9	4
	6–8	3500	400	15	40	0.2	13	1.1	1.0	1.0	4
	8–10	3500	400	15	40	0.3	15	1.2	1.1	1.2	5
Males	10–12	4500	400	20	40	0.4	17	1.3	1.3	1.4	5
	12–14	5000	400	20	45	0.4	18	1.4	1.4	1.6	5
	14–18	5000	400	25	55	0.4	20	1.5	1.5	1.8	5
	18–22	5000	400	30	60	0.4	18	1.6	1.4	2.0	5
	22–35	5000	–	30	60	0.4	18	1.7	1.4	2.0	5
	35–55	5000	–	30	60	0.4	17	1.7	1.3	2.0	5
	55–75+	5000	–	30	60	0.4	14	1.7	1.2	2.0	6
Females	10–12	4500	400	20	40	0.4	15	1.3	1.1	1.4	5
	12–14	5000	400	20	45	0.4	15	1.4	1.2	1.6	5
	14–16	5000	400	25	50	0.4	16	1.4	1.2	1.8	5
	16–18	5000	400	25	50	0.4	15	1.5	1.2	2.0	5
	18–22	5000	400	25	55	0.4	13	1.5	1.0	2.0	5
	22–35	5000	–	25	55	0.4	13	1.5	1.0	2.0	5
	35–55	5000	–	25	55	0.4	13	1.5	1.0	2.0	5
	55–75+	5000	–	25	55	0.4	13	1.5	1.0	2.0	6
Pregnancy		6000	400	30	60	0.8	15	1.8	+0.1	2.5	8
Lactation		8000	400	30	60	0.5	20	2.0	+0.5	2.5	6

* The allowance levels are intended to cover individual variations among most normal persons as they live in the USA under usual environmental stresses. The recommended allowances can be attained with a variety of common foods providing other nutrients for which human requirements have been less well defined.

** Entries on lines for age range 22–35 years represent the 'reference' man and woman at age 22 (see under 'Calories', page 493). All other entries represent allowances for the midpoint of the specified age range.

*** Assumes protein equivalent to human milk. For proteins not 100% utilized, factors should be increased proportionately.

† The folacin allowances refer to dietary sources as determined by *Lactobacillus casei* assay. Pure forms of folacin may be effective in doses less than a quarter of the recommended allowance.

†† Niacin equivalents include dietary sources of the vitamin itself plus 1 mEq for each 60 mg of dietary tryptophan.

Recommended daily calorie allowances (F
USA[2]) (Light physical activity, mean en

	Body weight (kg)	Body weight (lb)	RMR* at age 22
Men	50	110	1540
	55	121	1620
	60	132	1720
	65	143	1820
	70	154	1880
	75	165	1970
	80	176	2020
	85	187	2110
	90	198	2210
	95	209	2290
	100	220	2380
Women	40	88	1280
	45	99	1380
	50	110	1460
	55	121	1560
	58	128	1620
	60	132	1640
	65	143	1740
	70	154	1830

* RMR = resting metabolic rate, approxi
rate measured under basal conditions.

Energy requirements during various activi

Light work (2.5–4.9 kcal/min)	Moderately heavy work (5.0–7.4 kcal/min)
Light industrial and domestic work	Farm work
Gymnastics	Marching with pack
Tile-laying	Ballroom dancing
Painting	Playing tennis
Tending agricultural machines	Cycling
Driving goods vehicles	
Playing golf, bowling	

unreasonably heavy clothing is worn
creases with increasing environmen
tures between 30°C and 40°C the calc
0.5% per 1°C, but only when physica

The Food and Nutrition Board (
infants and children are average and a
in the case of adult allowances, they
accordance with observations of apj
growth as judged by amount of subc

The calorie allowances recommend
higher than those of the Food and Nut
of an assumed greater physical activit
ing various activities can be calculat
this page.

Protein

Dietary protein is the source of the
acids, i.e., those necessary for the synt
other nitrogenous substances (see the
protein allowance for adults recomme
tion Board (USA)[2] (page 494), based
for food protein, is 0.9 g protein per
day, a value about nine times the minim
Protein[4]. In the case of infants it is as
normal sufficient protein is obtained
though this provides little more thar
For the protein allowances recommer
pert Group see page 493. For premat

(For references see page 497)

Minerals

...phos-...orus (g)	Iodine (µg)	Iron (mg)	Mag-nesium (mg)
0.2	25	6	40
0.4	40	10	60
0.5	45	15	70
0.7	55	15	100
0.8	60	15	150
0.8	70	10	200
0.8	80	10	200
0.9	100	10	250
1.0	110	10	250
1.2	125	10	300
1.4	135	18	350
1.4	150	18	400
0.8	140	10	400
0.8	140	10	350
0.8	125	10	350
0.8	110	10	350
1.2	110	18	300
1.3	115	18	350
1.3	120	18	350
1.3	115	18	350
0.8	100	18	350
0.8	100	18	300
0.8	90	18	300
0.8	80	10	300
0.4	125	18	450
0.5	150	18	450

...le vitamins

...lavin g)	Thiamine (mg)	Vitamin B_6 (mg)	Vitamin B_{12} (µg)
	0.2	0.2	1.0
	0.4	0.3	1.5
	0.5	0.4	2.0
	0.6	0.5	2.0
	0.6	0.6	2.5
	0.7	0.7	3
	0.8	0.9	4
	1.0	1.0	4
	1.1	1.2	5
	1.3	1.4	5
	1.4	1.6	5
	1.5	1.8	5
	1.4	2.0	5
	1.4	2.0	5
	1.3	2.0	5
	1.2	2.0	6
	1.1	1.4	5
	1.2	1.6	5
	1.2	1.8	5
	1.2	2.0	5
	1.0	2.0	5
	1.0	2.0	5
	1.0	2.0	5
	1.0	2.0	6
	+0.1	2.5	8
	+0.5	2.5	6

) human milk. For proteins not 100% l proportionately.
lietary sources as determined by *Lacto-*lacin may be effective in doses less than vance.
· sources of the vitamin itself plus 1 mEq n.

Recommended daily calorie allowances (Food and Nutrition Board, USA[2]) (Light physical activity, mean environmental temperature 20 °C)

Body weight	(kg)	*(lb)*	RMR* at age 22	Age 22	Age 45	Age 65
Men	50	*110*	1540	2200	2000	1850
	55	*121*	1620	2350	2150	1950
	60	*132*	1720	2500	2300	2100
	65	*143*	1820	2650	2400	2200
	70	*154*	1880	2800	2600	2400
	75	*165*	1970	2950	2700	2500
	80	*176*	2020	3050	2800	2600
	85	*187*	2110	3200	2950	2700
	90	*198*	2210	3350	3100	2800
	95	*209*	2290	3500	3200	2900
	100	*220*	2380	3700	3400	3100
Women	40	*88*	1280	1550	1450	1300
	45	*99*	1380	1700	1550	1450
	50	*110*	1460	1800	1650	1500
	55	*121*	1560	1950	1800	1650
	58	*128*	1620	2000	1850	1700
	60	*132*	1640	2050	1900	1700
	65	*143*	1740	2200	2000	1850
	70	*154*	1830	2300	2100	1950

* RMR = resting metabolic rate, approximately 10% above the metabolic rate measured under basal conditions.

Energy requirements during various activities[7]

Light work (2.5–4.9 kcal/min)	Moderately heavy work (5.0–7.4 kcal/min)	Heavy work (7.5–9.9 kcal/min)	Very heavy work (over 10 kcal/min)
Light industrial and domestic work	Farm work	Mine-working	Felling trees
Gymnastics	Marching with pack	Playing football	Steelmaking
Tile-laying	Ballroom dancing		Swimming (crawl)
Painting	Playing tennis		Climbing
Tending agricultural machines	Cycling		
Driving goods vehicles			
Playing golf, bowling			

Normal energy requirements of recumbent adults[7]

Body weight kg / lb			45 / 99	50 / 110	55 / 121	60 / 132	65 / 143	70 / 154	75 / 165	80 / 176
Fat (%)	Body type: Men	Body type: Women	Energy requirement (kcal/min)							
5– 9	Lean	–	–	0.99	1.06	1.12	1.19	1.26	1.32	1.39
10–14	Average	–	–	0.94	1.01	1.08	1.14	1.21	1.28	1.34
15–19	Heavily built	Lean	0.82	0.89	0.96	1.03	1.09	1.16	1.23	1.30
20–24	Corpulent	Average	0.78	0.84	0.91	0.98	1.05	1.11	1.18	1.25
25–29	–	Heavily built	–	0.80	0.86	0.93	1.00	1.07	1.13	1.20
>30	–	Corpulent	–	–	0.81	0.88	0.95	1.02	1.08	1.15

Normal energy requirements of adults sitting and at rest[7]

Age in years	Number of subjects	Energy requirement (kcal/min) Mean	Range	s
Men (65 kg = 143 lb)				
20–39	30	1.39	0.97–1.79	0.25
40–64	30	1.37	0.87–1.94	0.29
65 and over	23	1.29	0.91–1.94	0.25
Women (55 kg = 121 lb)				
20–39	30	1.15	0.75–1.68	0.28
40–59	30	1.07	0.78–1.56	0.19
60 and over	23	1.09	0.77–1.62	0.31

Normal energy requirements of adults during walking[7]

Body weight kg / lb		45 / 99	55 / 121	65 / 143	75 / 165	85 / 187	95 / 209
Rate of walking km/h	Rate of walking mi/h	Energy requirement (kcal/min)					
3	*1.9*	2.1	2.5	2.8	3.2	3.5	3.8
4	*2.5*	2.7	3.2	3.6	4.0	4.3	4.6
5	*3.1*	3.2	3.7	4.2	4.7	5.1	5.5
6	*3.7*	3.8	4.4	4.9	5.4	5.9	6.4
7	*4.3*	4.4	5.0	5.5	6.1	6.6	7.1

unreasonably heavy clothing is worn. The calorie requirement increases with increasing environmental temperature; at temperatures between 30 °C and 40 °C the calorie intake should be raised by 0.5% per 1 °C, but only when physical activity is undiminished.

The Food and Nutrition Board (USA) calorie allowances for infants and children are average and approximate. Even more than in the case of adult allowances, they may need to be adjusted in accordance with observations of appetite, activity and nature of growth as judged by amount of subcutaneous fat.

The calorie allowances recommended by the FAO (page 493) are higher than those of the Food and Nutrition Board (USA) as a result of an assumed greater physical activity. Energy requirements during various activities can be calculated by means of the tables on this page.

Protein

Dietary protein is the source of the nitrogen and essential amino acids, i.e., those necessary for the synthesis of the body proteins and other nitrogenous substances (see the table opposite). The dietary protein allowance for adults recommended by the Food and Nutrition Board (USA)[2] (page 494), based on a utilization value of 70% for food protein, is 0.9 g protein per kilogramme body weight per day, a value about nine times the minimum requirement of Reference Protein[4]. In the case of infants it is assumed that when lactation is normal sufficient protein is obtained from the breast milk, even though this provides little more than the minimum requirement. For the protein allowances recommended by the FAO/WHO Expert Group see page 493. For premature infants the recommended allowance[6] is between 2.5 g and a maximum of 6 g per kilogramme body weight per day.

Carbohydrate and fat[2]

The desirable intake of carbohydrate and fat, like the optimal fatty-acid composition of foods, is difficult to assess. Apart from the body's specific needs for carbohydrate (e.g., brain energy), this and fat appear to be interchangeable as dietary energy sources, and in the body they are interconvertible, except that fatty acids with an even number of C-atoms from neutral fat, etc. cannot be used to form carbohydrate. Adaptation to diets very low in carbohydrate is possible, but for persons accustomed to a normal diet at least 100 g carbohydrate per day appears to be necessary if metabolic disturbances like ketosis, excessive protein breakdown, etc. are to be avoided. Normally, less than 1800 kcal of carbohydrate is stored as glycogen, so that if there is not enough in the diet it must be derived largely from dietary or body protein.

Dietary fat is also a carrier of other nutrients, including vitamins A, D, E and K. Calories, whether derived from dietary carbohydrate or fat, are stored mainly as fat in adipose cells. Except for the central nervous system, almost all body tissues utilize fatty acids directly as a source of energy.

The polyunsaturated fatty acids like linoleic and arachidonic acids have been shown to be essential for animals, and are very probably also essential for man. The minimum human requirements of the essential fatty acids should probably represent about 2% of the total calorie intake[9], or about 2.4 g linoleic acid per 1000 kcal of nutrient intake. In infants, a subclinical deficiency of linoleic acid

can be prevented by ensuring that the amount of this substance in the formula supplies 3% of the calories[10].

Essential amino acids

Of the 18 amino acids contained in food proteins 8 are essential in that the body is not capable of synthesizing them (tryptophan, phenylalanine, lysine, threonine, methionine, leucine, isoleucine, valine), 2 are semi-essential in that they are not synthesized in adequate amounts during growth (histidine, arginine), and 6 are non-essential in that the body can synthesize them from a nitrogen source such as any amino acid, ammonium salts or urea (aspartic acid, glutamic acid, proline, glycine, serine, alanine).

*Requirements of the essential amino acids**

Amino acid	Infants	Adults**		
	Minimum requirement[11] (mg/kg/day)	Minimum requirement		Recommended intake[12] (g/day)
		Young men[12] (g/day)	Young women[13] (g/day)	
L-Histidine	34	0	0	0
L-Tryptophan	22	0.25	0.16	0.50
L-Phenylalanine				
Tyrosine available†	90	0.30	0.22	–
Tyrosine not available	–	1.10	–	2.20
L-Lysine	103	0.80	0.50	1.60
L-Threonine	87	0.50	0.31	1.00
L-Methionine				
Cystine available††	45	0.20	0.35	–
Cystine not available	–	1.10	–	2.20
L-Leucine	150	1.10	0.62	2.20
L-Isoleucine	126	0.70	0.45	1.40
L-Valine	105	0.80	0.65	1.60

* Assuming that the nitrogen intake is adequate for the formation of the non-essential amino acids.
** The requirements are higher during pregnancy and lactation. The minimum requirement of men over 50 is higher than that of young men in respect of at least two amino acids (methionine 2.4–3.0 g/day, lysine 1.4–2.8 g/day)[14].
† 70–75% of the phenylalanine requirement can be met by tyrosine[12].
†† 80–90% of the methionine requirement can be met by cystine[12].

Water

The water requirement of the body is determined by the amount of heat it produces and by the load of solutes in the body fluids. It is closely linked to the intake of salt. The intake must replace water losses in the urine, faeces, sweat and insensible perspiration (skin and lungs). Under the most favourable conditions (low-solute diet, resting, no sweating) the total water supplied by the diet and metabolic processes should be at least 1.5 l/day[16]. A reasonable water allowance is 1 ml per calorie of food[2]. In hot, dry climates the water requirement can be considerably increased as a result of sweating. Under ordinary conditions, infants require proportionately more water than adults and should be given 1.5 ml per calorie of food[2].

See also 'Water and Electrolyte Balance', pages 523–530.

Sodium and chloride

The requirement of sodium and chloride is closely linked to the water balance of the body. Both the total body content and body-fluid concentration of sodium are homoeostatically controlled, moderate intakes being rapidly excreted in the urine while a reduction in intake causes excretion to drop quickly to a very low level[16]. Sodium deficiency is rare in healthy persons provided there is no abnormal loss. The normal recommended NaCl intake is 1 g per kilogramme of water[2]. Hard physical work in the tropics would require a daily intake of up to 19 g NaCl[17]. Normal diets in western Europe and USA contain 6–18 g NaCl.

Potassium

The minimum daily potassium requirement probably amounts to 0.8–1.3 g[2]. Normal diets in western countries provide 0.8–1.5 g potassium per 1000 kcal. An adequate potassium intake is important during prolonged intravenous feeding, recovery from severe diarrhoea, and diabetic acidosis; this should be met by an intake of 40–120 mg (1–3 mEq) per kg body weight per day.

Magnesium

The probable daily magnesium requirement[18] is 150 mg for children under 10 years and 200–300 mg for older children; with a daily protein intake of 70–80 g, men require 300–400, women 300 mg per day. The requirement seems to increase with increasing protein intake. For the recommendations of the Food and Nutrition Board (USA) see the table on page 494. An average diet provides 250–500 mg magnesium per day.

Calcium and phosphorus

Knowledge of the minimum requirements of calcium is inadequate, but it is well established that no injurious effects occur when the daily calcium intake lies between 300 mg and 2000 mg[19].

The desirable calcium intakes (*suggested practical allowances*) recommended by the FAO/WHO Expert Group (see table on page 493) are lower than those of the Food and Nutrition Board (USA)[2] (see table on page 494).

Although the calcium–phosphorus ratio in bone is 2:1 it is much lower in the soft tissues. This, and the fact that on a normal diet the intake of phosphorus always equals or exceeds that of calcium, have led to the recommendation[2] that the calcium and phosphorus allowances should be equal except in the case of young infants (see the table on page 494).

Iron

In order to remain in iron balance, the daily iron intake of men, as well as of women after the menopause, must be 0.5–1.0 mg; menstruating women require some 0.3–1.0 mg per day more[20]. The iron requirement is increased during pregnancy, particularly as a result of the increase in the total erythrocyte volume, and this need is met partly by mobilization of iron reserves. The latter are restored post partum when the total erythrocyte volume falls again.

During the first months of life the iron requirement of infants is met principally from endogenous sources. Special attention should be paid to the iron needs of growing girls, who have to meet not only the requirements of growth but also cover menstruation losses. Assuming that 10% of the dietary iron is absorbed, the following amounts must be available in the diet:

Dietary iron requirements (Committee on Iron Deficiency Anemia, American Medical Association[21])

	Iron requirement of body mg/day	Dietary iron content* mg/day
Men	0.5–1.0	5–10
Menstruating women	0.7–2.0	7–20
Pregnant women	2.0–4.8	20–48
Adolescents	1.0–2.0	10–20
Children	0.4–1.0	4–10
Infants	0.5–1.5	1.5 mg/kg**

* Assuming 10% absorption of dietary iron.
** Up to a maximum of 15 mg.

With the exception of those during pregnancy, these requirements are almost identical with the recommendation of the Food and Nutrition Board (USA) (page 494).

Estimates of dietary iron intake in the UK, USA, Australia and Canada have given values of 10–20 mg per person per day; various studies in the USA have shown that children aged 3–6 years have a daily iron intake of 3–11 mg[22].

In infants the iron requirement is not met by the normal intake of breast or cow's milk alone. Premature infants, as well as infants with iron deficiency, require additional iron from the 2nd to 3rd month of life on; this should be given in the form of enriched cereal products or possibly iron salts[23]. The iron requirement in pregnancy is likewise met only with difficulty from dietary sources.

Copper

Copper is an important component of various enzymes involved in oxygen transport. In USA and Europe the diet of adults contains an average of 1–5 mg copper per day[22]. The daily requirement of adults has been estimated at 1.5–2 mg[2, 22], that of infants and children at 0.04–0.14 mg per kilogramme body weight[24].

Manganese

Manganese is very probably an es
appears to be involved in the synthe
phosphorylation[25]. The daily diet co
an amount apparently sufficient to
order to maintain a positive manganes
of 0.2–0.3 mg manganese per kilogra
recommended for children[27].

Zinc

Zinc is an important component o
drogenases. The daily dietary intake
positive zinc balance is 10–15 mg[29]
milk falls during the course of lactati
inadequate to meet the infant's zinc r

Cobalt

Cobalt is a component of vitamin
not used by the human body in any
anaemia by cobalt salts is of doubtful

Molybdenum

Molybdenum is an essential com
particularly xanthine oxidase[31]. The n
sufficient molybdenum to meet the ne

Vanadium

Vanadium is possibly an essential
volved in lipid metabolism. The dail
2 mg[32].

Chromium

Chromium in its trivalent form is p
since there appears to be a relationsh
insulin function[33]. The daily diet cont
of which about 1% is absorbed, enou
quirement[34].

Selenium

Selenium has not been shown to be a
though a role in the metabolism of toc
taining sulphur has been reported[35].

Iodine

Iodine is a component of thyroid h
The minimum requirement of adults ha
per day; children and pregnant wor
ment[2, 36]. On the basis of the iodine tu
optimum requirement of adults has bee
day[37]. For the recommendations of th
(USA) see the table on page 494. The ic
with that of the soil from which the fo
tion of iodine to table salt in the propor
(as potassium iodide) is probably suffici
iodine intake[36]. On the average, iodi
following amounts of iodine (in µg,
Republic and Poland ≦ 3.8; Switzerlar
slavia 7.6; England and Wales 19; Arg
USA and Canada, international shippi

Fluorine

Fluorine is a component of dental e
protection against dental caries[39]. Wh
tains 1 mg fluoride per litre the inciden
in children aged 6–15 years, is less than
relatively poor in fluoride. With a drin
of 1 mg/l the daily fluoride intake has b
for adults and 0.4–0.8 mg for children a
Council on Nutrition has recommende
0.25 mg for infants and 0.5–1.0 mg for
In areas where the fluoride content o

dosis; this should be met by an intake of
kg body weight per day.

gnesium requirement[16] is 150 mg for
d 200–300 mg for older children; with
70–80 g, men require 300–400, women
ement seems to increase with increasing
ommendations of the Food and Nutri-
able on page 494. An average diet pro-
um per day.

num requirements of calcium is inade-
hed that no injurious effects occur when
s between 300 mg and 2000 mg[19].
ntakes (*suggested practical allowances*) re-
WHO Expert Group (see table on page
the Food and Nutrition Board (USA)[2]

osphorus ratio in bone is 2:1 it is much
his, and the fact that on a normal diet
ways equals or exceeds that of calcium,
ition[2] that the calcium and phosphorus
except in the case of young infants (see

a balance, the daily iron intake of men,
the menopause, must be 0.5–1.0 mg;
re some 0.3–1.0 mg per day more[20].
reased during pregnancy, particularly
the total erythrocyte volume, and this
ization of iron reserves. The latter are
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.....	0.4–1.0	4–10
.....	0.5–1.5	1.5 mg/kg**

dietary iron.

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page 494).
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nent is not met by the normal intake
Premature infants, as well as infants
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h difficulty from dietary sources.

ponent of various enzymes involved
nd Europe the diet of adults contains
per day[22]. The daily requirement of
5–2 mg[2, 22], that of infants and chil-
gramme body weight[24].

Manganese

Manganese is very probably an essential nutrient. The element appears to be involved in the synthesis of lipids and in oxidative phosphorylation[26]. The daily diet contains 2–5 mg manganese[26], an amount apparently sufficient to meet human requirements. In order to maintain a positive manganese balance, daily dietary intakes of 0.2–0.3 mg manganese per kilogramme body weight have been recommended for children[27].

Zinc

Zinc is an important component of enzymes, particularly dehydrogenases. The daily dietary intake necessary for maintaining a positive zinc balance is 10–15 mg[28]. The zinc content of breast milk falls during the course of lactation (see page 688) and may be inadequate to meet the infant's zinc requirement[29].

Cobalt

Cobalt is a component of vitamin B_{12} and as far as is known is not used by the human body in any other way. The treatment of anaemia by cobalt salts is of doubtful value[30].

Molybdenum

Molybdenum is an essential component of various enzymes, particularly xanthine oxidase[31]. The normal diet appears to contain sufficient molybdenum to meet the need for this element.

Vanadium

Vanadium is possibly an essential nutrient in that it may be involved in lipid metabolism. The daily dietary intake amounts to 2 mg[32].

Chromium

Chromium in its trivalent form is probably an essential nutrient since there appears to be a relationship between this element and insulin function[33]. The daily diet contains 30–100 µg of chromium, of which about 1% is absorbed, enough to meet any possible requirement[34].

Selenium

Selenium has not been shown to be a significant nutrient for man, though a role in the metabolism of tocopherol and amino acids containing sulphur has been reported[35].

Iodine

Iodine is a component of thyroid hormones (see pages 725 sq.). The minimum requirement of adults has been estimated at 50–75 µg per day; children and pregnant women have a higher requirement[2, 36]. On the basis of the iodine turnover of the whole body the optimum requirement of adults has been estimated at 114–357 µg per day[37]. For the recommendations of the Food and Nutrition Board (USA) see the table on page 494. The iodine content of foods varies with that of the soil from which the food has originated. The addition of iodine to table salt in the proportion of 1:10000 to 1:100000 (as potassium iodide) is probably sufficient to guarantee an adequate iodine intake[38]. On the average, iodinated table salt contains the following amounts of iodine (in µg/g NaCl): German Federal Republic and Poland ≦ 3.8; Switzerland, Netherlands, Italy, Jugoslavia 7.6; England and Wales 19; Argentina 25; New Zealand 38; USA and Canada, international shipping and aircraft 76.

Fluorine

Fluorine is a component of dental enamel and plays a role in its protection against dental caries[39]. When the drinking water contains 1 mg fluoride per litre the incidence of dental caries, especially in children aged 6–15 years, is less than when the drinking water is relatively poor in fluoride. With a drinking-water fluoride content of 1 mg/l the daily fluoride intake has been estimated at 1.4–1.8 mg for adults and 0.4–0.8 mg for children aged 1–3 years. The Canadian Council on Nutrition has recommended a daily fluoride intake of 0.25 mg for infants and 0.5–1.0 mg for children aged 1–14 years[40]. In areas where the fluoride content of the natural water is low, fluoridation of the drinking water, in the proportion 1:1000000, is recommended by the Food and Nutrition Board (USA)[2], though the desirability of this measure is still internationally debated.

Vitamins

Vitamin requirements are dealt with under the individual vitamins in the chapter 'Vitamins', pages 457–492. The recommendations of the FAO/WHO Expert Group regarding vitamin A, thiamine, riboflavin and niacin and of the Food and Nutrition Board (USA) regarding these and other important vitamins are shown in the tables on page 493 and 494 respectively.

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